Vaccine 35 (2017) 1926–1935

Contents lists available at ScienceDirect

Vaccine

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

A randomized study of fever prophylaxis and the immunogenicity of routine pediatric vaccinations

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ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 3 February 2017
Accepted 14 February 2017
Available online 3 March 2017

Keywords:
Pneumococcal conjugate vaccine
Immune response
Antipyretic
Ibuprofen
Paracetamol
Infant

ABSTRACT

Objective: Prophylactic antipyretic use during pediatric vaccination is common. This study assessed whether paracetamol or ibuprofen prophylaxis interfere with immune responses to the 13-valent pneumococcal conjugate vaccine (PCV13) given concomitantly with the combined DTaP/HBV/IPV/Hib vaccine.
Methods: Subjects received prophylactic paracetamol or ibuprofen at 0, 6–8, and 12–16 h after vaccination, or 6–8 and 12–16 h after vaccination at 2, 3, 4, and 12 months of age. At 5 and 13 months, immune responses were evaluated versus responses in controls who received no prophylaxis.
Results: After the infant series, paracetamol recipients had lower levels of circulating serotype-specific pneumococcal anticapsular immunoglobulin G than controls, reaching significance ($P < 0.0125$) for 5 serotypes (serotypes 3, 4, 5, 6B, and 23F) when paracetamol was started at vaccination. Opsonophagocytic activity assay (OPA) results were similar between groups. Ibuprofen did not affect pneumococcal responses, but significantly ($P < 0.0125$) reduced antibody responses to pertussis filamentous hemagglutinin and tetanus antigens after the infant series when started at vaccination. No differences were observed for any group after the toddler dose.
Conclusions: Prophylactic antipyretics affect immune responses to vaccines; these effects vary depending on the vaccine, antipyretic agent, and time of administration. In infants, paracetamol may interfere with

Abbreviations: AE, adverse event; DTaP/HBV/IPV/Hib, diphtheria-tetanus-acellular pertussis, hepatitis B, inactivated poliovirus, and *H influenzae* type b (INFANRIX® hexa); FHA, filamentous hemagglutinin; GMC, geometric mean concentration; GMR, geometric mean ratio; GMT, geometric mean titer; IgG, immunoglobulin G; LLOQ, lower limit of quantitation; mITT, modified intent to treat; OPA, opsonophagocytic activity; PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; SAE, serious adverse event.

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http://dx.doi.org/10.1016/j.vaccine.2017.02.035
0264-410X/

immune responses to pneumococcal antigens, and ibuprofen may reduce responses to pertussis and tetanus antigens. The use of antipyretics for fever prophylaxis during infant vaccination merits careful consideration.

Conclusions: ClinicalTrials.gov identifier: NCT01392378 https://clinicaltrials.gov/ct2/show/NCT01392378?term=NCT01392378&rank=1

1. Introduction

Immunization against *Streptococcus pneumoniae* with a pneumococcal conjugate vaccine (PCV) is routinely recommended for children in many countries. Fever is frequently associated with pediatric vaccination, and over-the-counter antipyretics are often administered prophylactically at vaccination or shortly thereafter. While paracetamol may reduce fever [1,2] and other common adverse effects [3,4] after vaccination, limited data suggest that it may also have deleterious effects on immune response. A 2009 study [5] reported decreased antibody production against all 10 pneumococcal serotypes in infants given paracetamol concomitantly with 10-valent PCV vaccination. Moreover, immune responses to a coadministered multicomponent vaccine were also reduced; some of these effects persisted 1 month after a booster dose for both vaccines [5]. Limitations of that study included evaluation of only one antipyretic agent (paracetamol) given in a single dosing regimen; other agents and the impact of dose timing on vaccine immune responses were not explored. Ibuprofen is also available over-the-counter and is widely used in this setting [6–9], but no information exists regarding its effect, if any, on the immunogenicity of routinely administered vaccines.

This paper reports results of a large, randomized, controlled, open-label trial examining effects of coadministration or delayed administration (ie, dose timing) of paracetamol or ibuprofen on immune responses to PCV13 and coadministered antigens after an infant vaccination series and a toddler dose.

2. Methods

This research protocol (ClinicalTrials.gov identifier: NCT01392378) sponsored by Pfizer Inc was reviewed and approved by institutional review boards and/or independent ethics committees for each participating center. This study was conducted according to principles derived from the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. Both parents of all participants gave written, informed consent before enrollment and before performance of study-related procedures. Data analysis was performed by the sponsor.

2.1. Objectives

The primary objective was to assess the effect of prophylactic paracetamol or ibuprofen on the immunogenicity of PCV13 (Prevnar 13/Prevenar 13®, Pfizer Inc, Sandwich, United Kingdom) relative to controls, as measured by serotype-specific immunoglobulin G (IgG) geometric mean concentrations (GMCs) after completion of an infant vaccination series. Secondary objectives included assessment of prophylactic antipyretic effects on PCV13 serotype-specific IgG GMCs after a toddler dose, PCV13 immunogenicity measured by serotype-specific opsonophagocytic activity (OPA) geometric mean titers (GMTs) in a subset of subjects after the infant series, and immunogenicity of diphtheria-tetanus-acellular pertussis, hepatitis B, inactivated poliovirus, and *H influenzae* type b (DTaP/HBV/IPV/Hib; INFANRIX® hexa, GlaxoSmithKline, Rixensart, Belgium) antigens measured by GMCs and GMTs after the infant series and toddler dose. The PCV13 safety profile was evaluated by measuring fever incidence and adverse events (AEs).

2.2. Study design

In this study conducted from August 2011–January 2013, subjects from 14 sites in Poland were enrolled and randomized by an interactive voice response system into 5 groups (10:10:10:10:12) to receive prophylactic antipyretics with PCV13 and DTaP/HBV/IPV/Hib at approximately 2, 3, 4 (infant series), and 12 months (toddler dose) of age. At each vaccine visit, Groups 1 and 2 received paracetamol (15 mg/kg/dose) or ibuprofen (10 mg/kg/dose), respectively, starting 6–8 h after vaccination and again 6–8 h after the initial antipyretic dose. Groups 3 and 4 received the same respective doses as Groups 1 and 2, but began paracetamol (Group 3) or ibuprofen (Group 4) with vaccination. Controls (Group 5) did not receive prophylactic antipyretics. For all groups, antipyretics were permitted for treatment of fever or other symptoms at the treating investigator's discretion. All antipyretic doses, including missed or additional doses, were recorded in an electronic diary (e-diary).

At approximately 5 and 13 months of age, blood samples (~5 mL) were collected for assessing serum concentrations of anticapsular IgG for all 13 pneumococcal serotypes in the vaccine by standardized ELISA, which used a C polysaccharide-containing cell wall extract and serotype 22F capsular polysaccharide [10–12]. The same blood samples were used to assess DTaP/HBV/IPV/Hib antibody responses for all subjects and serum OPA for the 13 serotypes (described in [13]) in a randomly selected subset of 75 subjects per group. Antibody responses to DTaP/HBV/IPV/Hib were measured as previously described [14–16].

Rectal temperature, recorded in the e-diary, was measured 6–8 h postvaccination, 6–8 h later, and during the next 3 days at bedtime and when fever was suspected. Fever was defined as a rectal temperature of 38.0–39.0 °C (mild), 39.1–40.0 °C (moderate), and >40.0 °C (severe). AEs and serious AEs (SAEs) were collected throughout the study in a case report form, and were analyzed as percentages of each group reporting a specific MedDRA preferred term.

2.3. Vaccines administered

PCV13 (lot number 10-088269) and DTaP/HBV/IPV/Hib (lot numbers 11-001342, 11-002167, 11-008164, or 11-008296) were given intramuscularly in the anterolateral thigh muscle in opposing legs. PCV13 contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated to CRM_{197}. Each 0.5-mL dose contains 4.4 μg of serotype 6B, 2.2 μg each of the remaining 12 saccharides, 5 mM succinate buffer, 0.02% polysorbate 80, and 0.125 mg aluminium phosphate. Each 0.5-mL dose of DTaP/HBV/IPV/Hib contains 25 Lf diphtheria toxoid, 10 Lf tetanus toxoid, 25 μg pertussis toxin, 25 μg filamentous haemagglutinin (FHA), 8 μg pertactin, 10 μg hepatitis

B surface antigen, 40 D-antigen units (DU) of type 1 poliovirus, 8 DU type 2 poliovirus, 32 DU type 3 poliovirus, 10 μg Hib capsular polysaccharide covalently bound to 25 μg tetanus toxoid, 12.6 mg lactose, 4.5 mg sodium chloride, 0.7 mg aluminium adjuvants (as salts), and 0.12 mg aluminium phosphate [17].

2.4. Inclusion and exclusion criteria

Eligible subjects were 2 months of age (≥56 to ≤98 days) and had not previously received pneumococcal vaccine or DTaP/HBV/IPV/Hib. Other routine vaccinations were permitted throughout the study. Exclusion criteria included: contraindication to vaccination with either study vaccine; history of anaphylactic reaction to any vaccine or vaccine-related component; allergy or contraindication to paracetamol or ibuprofen; and chronic use of medications with known interactions with either antipyretic.

2.5. Immunogenicity and safety endpoints

The immunogenicity endpoints of this study were: serotype-specific IgG GMCs and geometric mean ratios (GMRs) relative to controls for 13 pneumococcal serotypes measured 1 month after the infant series and toddler dose; the proportion of subjects achieving a serotype-specific IgG antibody concentration ≥0.35 μg/mL for the 13 pneumococcal serotypes 1 month after the infant series and toddler dose; serotype-specific OPA GMTs and GMRs relative to controls for the 13 pneumococcal serotypes 1 month after the infant series in a subset of subjects; the proportion of study participants achieving a serotype-specific OPA ≥ the lower limit of quantitation (LLOQ) for the 13 pneumococcal serotypes 1 month after the infant series; DTaP/HBV/IPV/Hib GMCs and GMRs relative to controls after the infant series and toddler dose; and the proportion of subjects achieving prespecified antibody levels to DTaP/HBV/IPV/Hib antigens 1 month after the infant series and toddler dose. Seroprotective IgG GMC thresholds were defined as 0.35 μg/mL for the 13 pneumococcal serotypes [18], and as follows for the DTaP/HBV/IPV/Hib antigens: Hib (PRP; ≥0.15 μg/mL and ≥1.0 μg/mL); diphtheria (≥0.1 IU/mL); pertussis (PT, FHA, PRN; 5 EL. U/mL, ≥5th percentile in Group 5); tetanus (≥0.1 IU/mL); HBV (≥10 mIU/mL); poliovirus (≥1:8 titer) [17].

Safety endpoints were fever, antipyretic use, AEs, and SAEs.

2.6. Statistical analyses

The modified intent-to-treat (mITT) population was the primary population for statistical analyses and comprised subjects who had no major protocol violations, received the randomized antipyretic regimen after all vaccinations, may have received additional doses of antipyretics (for fever), and had blood drawn within study-specified periods. The per-protocol population comprised subjects included in the mITT population but had received no antipyretics beyond those specified by the protocol. GMCs and GMRs were estimated by serotype using a general linear model with a fixed effect of assigned antipyretic regimen group and natural log-transformed IgG data as the response; a similar approach was taken for each of the vaccine antigens contained in DTaP/HBV/IPV/Hib and for GMTs. All subjects who received ≥ 1 dose of study vaccine were included in safety analyses, which were performed separately for each dose.

As 4 between-group comparisons were of interest (each of Groups 1–4 relative to Group 5), it was necessary to control the type 1 error (false-positive) rate for multi-group comparisons. Statistical significance for each of the between-group comparisons therefore required a P value of <0.0125 (i.e., Bonferroni adjustment) to maintain the overall experiment-wise error rate at 5%. To also account for the 13 multiple comparisons across serotypes within each between-group comparison, the Benjamini-Hochberg false discovery rate testing procedure [19] was applied to adjust for multiplicity within each between-group comparison across serotypes. Using the false discovery rate procedure, 150 evaluable subjects per arm were estimated to provide ≥90% power to detect 50% decreases in GMRs using these statistical adjustments. Similar comparisons were constructed for concomitant antigens; however, as these comparisons were secondary/exploratory, no type 1 error adjustment was applied.

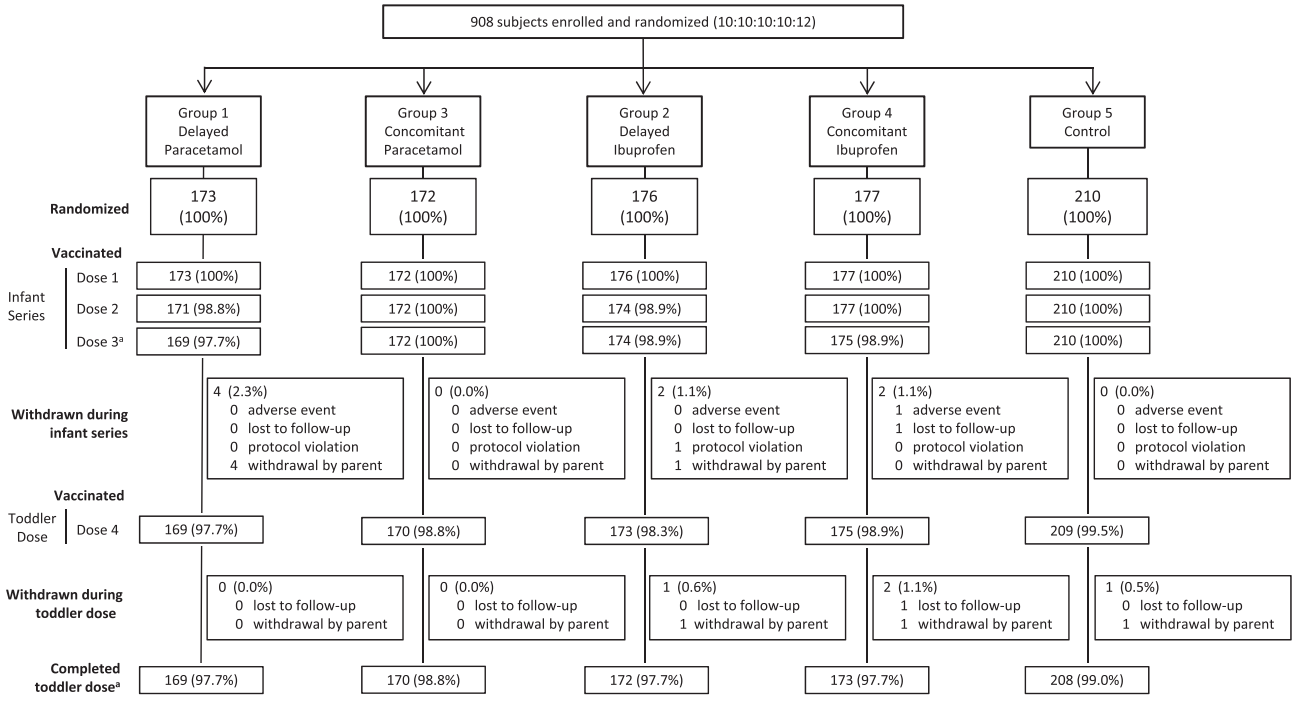

Fig. 1. Subject Disposition. [a]Completion of this dose includes blood draw.

Table 1
IgG GMCs 1 month after the infant series and toddler dose, including ratios relative to controls (mITT immunogenicity population).

Serotype	After infant series				After toddler dose			
Group[a]	N[b]	GMC[c] (95% CI)[d]	Ratio (95% CI)[d]	*P*-value[e]	N[b]	GMC[c] (95% CI)[d]	Ratio (95% CI)[d]	*P*-value[e]
PCV7 Serotypes								
4								
Group 1	137	1.64 (1.44–1.87)	0.81 (0.69–0.96)	0.0856	130	3.07 (2.66–3.54)	0.99 (0.82–1.19)	0.9350
Group 3	148	1.48 (1.31–1.68)	0.73 (0.62–0.86)**	0.0012	143	2.97 (2.60–3.41)	0.96 (0.80–1.14)	0.6922
Group 2	155	1.99 (1.76–2.25)	0.99 (0.84–1.16)	0.8546	143	3.43 (3.00–3.93)	1.11 (0.93–1.32)	0.7918
Group 4	146	2.07 (1.82–2.34)	1.02 (0.87–1.20)	0.8414	139	3.43 (2.99–3.94)	1.11 (0.93–1.32)	0.9765
Group 5	210	2.02 (1.82–2.25)			206	3.10 (2.77–3.48)		
6B								
Group 1	136	0.68 (0.55–0.83)	0.83 (0.64–1.08)	0.2412	130	6.70 (5.76–7.78)	0.95 (0.78–1.15)	0.6915
Group 3	148	0.56 (0.46–0.68)	0.69 (0.53–0.88)**	0.0093	143	6.38 (5.53–7.36)	0.90 (0.75–1.09)	0.4389
Group 2	155	0.91 (0.76–1.10)	1.13 (0.88–1.44)	0.4548	144	8.01 (6.94–9.24)	1.13 (0.94–1.36)	0.7918
Group 4	146	0.90 (0.74–1.09)	1.10 (0.86–1.42)	0.8414	139	7.30 (6.31–8.44)	1.03 (0.85–1.24)	0.9765
Group 5	210	0.81 (0.69–0.96)			206	7.08 (6.28–7.98)		
9V								
Group 1	138	1.13 (1.01–1.27)	0.87 (0.75–1.00)	0.1749	130	2.15 (1.93–2.40)	0.99 (0.87–1.14)	0.9350
Group 3	148	1.17 (1.05–1.30)	0.89 (0.78–1.03)	0.1351	143	2.17 (1.96–2.41)	1.00 (0.88–1.15)	0.9430
Group 2	155	1.45 (1.30–1.61)	1.11 (0.96–1.27)	0.3121	144	2.23 (2.01–2.47)	1.03 (0.90–1.18)	0.7918
Group 4	147	1.40 (1.26–1.56)	1.07 (0.93–1.24)	0.8414	139	2.12 (1.91–2.35)	0.98 (0.85–1.12)	0.9765
Group 5	210	1.31 (1.19–1.43)			206	2.16 (1.99–2.36)		
14								
Group 1	138	4.45 (3.76–5.26)	0.83 (0.67–1.03)	0.2158	130	8.10 (7.04–9.31)	0.89 (0.74–1.06)	0.5167
Group 3	148	4.75 (4.04–5.58)	0.88 (0.72–1.09)	0.2472	143	7.95 (6.96–9.08)	0.87 (0.73–1.04)	0.2514
Group 2	155	4.73 (4.04–5.54)	0.88 (0.71–1.08)	0.3279	144	8.40 (7.36–9.59)	0.92 (0.78–1.10)	0.7918
Group 4	147	5.26 (4.48–6.19)	0.98 (0.79–1.21)	0.8414	139	9.12 (7.96–10.43)	1.00 (0.84–1.19)	0.9872
Group 5	210	5.38 (4.70–6.16)			206	9.10 (8.15–10.17)		
18C								
Group 1	138	1.47 (1.29–1.66)	0.95 (0.81–1.12)	0.6193	130	1.35 (1.20–1.53)	0.85 (0.73–1.00)	0.2582
Group 3	148	1.25 (1.11–1.42)	0.82 (0.70–0.96)	0.0191	143	1.36 (1.21–1.53)	0.86 (0.74–1.00)	0.2514
Group 2	155	1.73 (1.54–1.95)	1.13 (0.96–1.32)	0.3121	144	1.68 (1.49–1.88)	1.06 (0.91–1.23)	0.7918
Group 4	147	1.75 (1.55–1.97)	1.14 (0.97–1.33)	0.8414	139	1.63 (1.45–1.84)	1.03 (0.88–1.20)	0.9765
Group 5	210	1.54 (1.39–1.70)			206	1.59 (1.44–1.75)		
19F								
Group 1	138	1.78 (1.57–2.02)	0.90 (0.76–1.05)	0.2412	130	8.41 (7.17–9.87)	1.06 (0.86–1.30)	0.6915
Group 3	148	1.59 (1.41–1.80)	0.80 (0.68–0.94)	0.0135	143	7.53 (6.47–8.76)	0.95 (0.78–1.15)	0.6922
Group 2	155	2.30 (2.04–2.59)	1.15 (0.99–1.35)	0.3121	144	8.99 (7.72–10.46)	1.13 (0.93–1.38)	0.7918
Group 4	147	2.04 (1.81–2.31)	1.03 (0.87–1.21)	0.8414	139	8.02 (6.88–9.36)	1.01 (0.83–1.23)	0.9872
Group 5	210	1.99 (1.80–2.20)			206	7.95 (7.00–9.02)		
23F								
Group 1	137	0.85 (0.72–1.00)	0.81 (0.66–1.00)	0.1749	130	2.34 (2.01–2.73)	0.85 (0.70–1.04)	0.3609
Group 3	148	0.73 (0.62–0.86)	0.70 (0.57–0.86)**	0.0038	142	2.37 (2.05–2.75)	0.86 (0.71–1.05)	0.2514
Group 2	155	1.19 (1.02–1.40)	1.15 (0.93–1.41)	0.3121	144	2.96 (2.56–3.43)	1.08 (0.89–1.30)	0.7918
Group 4	146	1.07 (0.91–1.26)	1.03 (0.84–1.27)	0.8414	139	2.86 (2.47–3.32)	1.04 (0.86–1.26)	0.9765
Group 5	210	1.04 (0.91–1.19)			206	2.75 (2.43–3.10)		
Additional Serotypes								
1								
Group 1	138	1.12 (0.98–1.27)	0.90 (0.76–1.06)	0.2412	130	2.80 (2.47–3.17)	0.92 (0.79–1.08)	0.6304
Group 3	148	1.02 (0.90–1.16)	0.82 (0.70–0.96)	0.0241	143	2.66 (2.36–3.00)	0.88 (0.75–1.03)	0.2514
Group 2	155	1.50 (1.33–1.69)	1.20 (1.02–1.41)	0.2053	144	3.22 (2.85–3.62)	1.06 (0.91–1.24)	0.7918
Group 4	147	1.29 (1.14–1.47)	1.04 (0.88–1.22)	0.8414	139	3.12 (2.76–3.52)	1.03 (0.88–1.20)	0.9765
Group 5	210	1.25 (1.12–1.38)			206	3.04 (2.75–3.35)		
3								
Group 1	138	0.71 (0.63–0.79)	0.81 (0.69–0.94)	0.0732	129	0.46 (0.40–0.52)	0.84 (0.71–1.01)	0.2582
Group 3	148	0.57 (0.51–0.64)	0.65 (0.56–0.76)***	<0.0001	143	0.46 (0.40–0.52)	0.85 (0.72–1.01)	0.2514
Group 2	155	0.83 (0.75–0.93)	0.95 (0.82–1.10)	0.6090	144	0.54 (0.47–0.61)	0.99 (0.84–1.18)	0.9279
Group 4	147	0.84 (0.75–0.94)	0.96 (0.82–1.11)	0.8414	138	0.49 (0.42–0.55)	0.90 (0.76–1.07)	0.9765
Group 5	210	0.88 (0.79–0.96)			203	0.54 (0.48–0.60)		
5								
Group 1	137	0.79 (0.69–0.91)	0.98 (0.82–1.16)	0.7828	130	2.33 (2.07–2.63)	0.82 (0.71–0.96)	0.1424
Group 3	148	0.63 (0.55–0.72)	0.77 (0.65–0.92)**	0.0093	143	2.40 (2.15–2.69)	0.85 (0.73–0.98)	0.2514
Group 2	155	0.98 (0.86–1.11)	1.12 (1.02–1.43)	0.2053	144	2.75 (2.46–3.07)	0.97 (0.84–1.12)	0.7918
Group 4	146	0.90 (0.78–1.02)	1.11 (0.93–1.31)	0.8414	139	2.62 (2.33–2.93)	0.92 (0.79–1.07)	0.9765
Group 5	210	0.81 (0.73–0.91)			206	2.84 (2.59–3.12)		
6A								
Group 1	138	0.97 (0.84–1.13)	0.88 (0.73–1.07)	0.2412	130	5.12 (4.48–5.86)	0.93 (0.78–1.10)	0.6304
Group 3	148	0.85 (0.74–0.98)	0.77 (0.64–0.93)	0.0135	143	5.27 (4.64–5.99)	0.95 (0.81–1.13)	0.6922
Group 2	155	1.25 (1.09–1.44)	1.14 (0.95–1.37)	0.3121	144	5.73 (5.04–6.50)	1.04 (0.88–1.22)	0.7918
Group 4	146	1.22 (1.06–1.41)	1.11 (0.92–1.34)	0.8414	139	5.36 (4.70–6.10)	0.97 (0.82–1.15)	0.9765
Group 5	210	1.10 (0.97–1.24)			206	5.52 (4.97–6.14)		

(*continued on next page*)

Table 1 (*continued*)

Serotype	After infant series				After toddler dose			
Group[a]	N[b]	GMC[c] (95% CI)[d]	Ratio (95% CI)[d]	*P*-value[e]	N[b]	GMC[c] (95% CI)[d]	Ratio (95% CI)[d]	*P*-value[e]
7F								
Group 1	138	1.94 (1.74–2.16)	0.90 (0.79–1.03)	0.2412	130	3.79 (3.42–4.19)	0.95 (0.84–1.08)	0.6572
Group 3	148	1.83 (1.65–2.03)	0.85 (0.75–0.98)	0.0275	142	3.56 (3.23–3.92)	0.89 (0.79–1.01)	0.2514
Group 2	155	2.22 (2.01–2.46)	1.03 (0.91–1.18)	0.6652	144	3.89 (3.54–4.28)	0.98 (0.86–1.11)	0.7918
Group 4	146	2.28 (2.06–2.53)	1.06 (0.93–1.21)	0.8414	139	3.97 (3.60–4.38)	1.00 (0.88–1.13)	0.9872
Group 5	210	2.15 (1.97–2.34)			206	3.98 (3.67–4.31)		
19A								
Group 1	137	2.70 (2.38–3.07)	0.90 (0.76–1.06)	0.2412	129	7.11 (6.22–8.12)	0.92 (0.78–1.09)	0.6304
Group 3	148	2.53 (2.24–2.86)	0.84 (0.72–0.99)	0.0387	142	7.31 (6.43–8.30)	0.95 (0.80–1.12)	0.6922
Group 2	155	3.39 (3.01–3.82)	1.13 (0.96–1.32)	0.3121	144	7.99 (7.04–9.06)	1.04 (0.88–1.22)	0.7918
Group 4	146	3.14 (2.77–3.55)	1.04 (0.89–1.22)	0.8414	139	7.35 (6.47–8.36)	0.95 (0.81–1.13)	0.9765
Group 5	210	3.02 (2.72–3.34)			206	7.71 (6.94–8.57)		

Note: P values for comparison of the antipyretic groups with control are adjusted using the false discovery rate procedure.
Abbreviations: CI = confidence interval; GMC = Geometric LSMean Concentration; IgG = immunoglobulin G; LSMean = least squares mean; mITT = modified intent-to-treat.
** P < 0.0125.
*** P < 0.001.
[a] Group 1, delayed paracetamol; Group 3, concomitant paracetamol; Group 2, delayed ibuprofen; Group 4, concomitant ibuprofen; Group 5, control.
[b] N = number of subjects with a determinate IgG concentration to the given serotype.
[c] GMCs were calculated using all subjects with available data for the specified blood draw.
[d] CIs are back transformations of CIs based on analysis of log-transformed IgG values using ANOVA with the antipyretic regimen group as a fixed effect. The ratio and related CIs are back transformed from the difference of the LSMean of a specific antipyretic group minus the LSMean of controls.
[e] *P*-values are adjusted using the false discovery rate procedure.

With a dropout rate of approximately 15% and a 10:10:10:10:12 randomization ratio, 908 subjects needed to be enrolled. Although the dropout rate for Group 5 was the same as for the other groups, to achieve 150 evaluable subjects, additional subjects were recruited for Group 5 with the expectation that some subjects would receive antipyretics as per standard recommendation.

3. Results

3.1. Subjects and immunogenicity

Between August 2011 and January 2013, 908 subjects were enrolled and randomized into 1 of 5 groups. Groups 1 and 3 received paracetamol (delayed and concomitant, respectively), and Groups 2 and 4 received ibuprofen (delayed and concomitant, respectively). Group 5 received no prophylactic antipyretics (control) (Fig. 1). Nine hundred (99.1%) subjects completed the infant vaccination series and blood draw, and 892 (98.2%) completed the toddler vaccination dose and blood draw (Fig. 1). The infant series mITT population included 800 (88.1%) subjects whose mean age was 65.7 ± 9.6 days at dose 1, and 47% of whom were female; all study groups were demographically similar (Table S1). Fewer than 10% of subjects in any group missed any doses of protocol-specified antipyretics (Table S2).

After the infant series, pneumococcal IgG GMCs among Groups 1 and 3 were lower than Group 5 for all serotypes, reaching statistical significance (P < 0.0125) in Group 3 for 5 of 13 serotypes (serotypes 3, 4, 5, 6B, and 23F) (Table 1). IgG GMCs in Groups 2 and 4 were not significantly different from Group 5 (Table 1). There were no significant differences in IgG GMCs among groups after the toddler dose (Table 1). Similarly, no significant differences were observed in the percentage of subjects in any group who achieved the prespecified level of serotype-specific pneumococcal IgG (≥0.35 μg/mL) for the infant and toddler mITT populations (Tables S3 and S4).

In the subset of subjects in which functional antibody responses were assessed, pneumococcal OPA GMTs after the infant series were not significantly different for any antipyretic group versus controls, although a slight numerical reduction was observed in Group 3 (Table S5). After the infant series, the majority of participants in each group (mITT population) achieved an OPA level ≥ LLOQ for each serotype except serotype 1 (Table S6).

Immune responses to DTaP/HBV/IPV/Hib antigens were assessed 1 month after the infant series and toddler dose. IgG geometric means for anti-pertussis FHA and anti-tetanus antibodies after the infant series were significantly (P < 0.0125) lower in Group 4 compared with Group 5 (pertussis FHA ratio, 0.73 [95% CI, 0.64–0.85], and tetanus, 0.74 [95% CI, 0.63–0.87]), but Groups 1, 3, and 2 showed no significant differences for any DTaP/HBV/IPV/Hib antigens (Table 2). There were no statistically significant differences in geometric means between Groups 1–4 and Group 5 for any of these antigens after the toddler dose (Table 2). The majority of subjects also achieved the prespecified level of antibody for each antigen after the infant series and toddler dose (Table S7).

3.2. Safety endpoints for the infant series and toddler dose

For all groups, fever was generally mild and of short duration (mean ≤ 1.5 days). In Group 5, approximately 10–20% of subjects reported fever on days 1 or 2 after any dose (Fig. 2A), and approximately 5–10% received an antipyretic agent to treat fever (Fig. 2B). At any vaccine dose, Groups 1 and 2 (delayed treatment) had more fever on day 1 than Groups 3 and 4, with the percentage of subjects reporting fever increasing on day 2 in Group 2. Subjects in Group 2 reported more fever on day 2 than Group 1 (Fig. 2A). Relatively few subjects in Groups 3 and 4 experienced fever on day 1. After all doses, day 2 fever rates were higher among Groups 2 and 4 (range, 17.3–41.0%) compared with Groups 1 and 3 (range, 11.8–26.8%) or Group 5 (range, 13.2–21.9%) (Fig. 2A).

Adverse events reflected events common among the study population and were numerically similar across groups during the infant series (35–40%) and after the toddler dose (15–20%). The number of subjects reporting SAEs during the study were similar across groups, ranging from 3–11 (1.7–6.4%) during the infant series, 6–14 (3.5–8.0%) after the infant series, and 1–3 (0.5–1.8%) after the toddler dose. All SAEs resolved, and none were considered by the investigator to be related to study vaccine. Only 1 subject withdrew from the study due to an AE. The event (somnolence) was considered related to ibuprofen and subsequently resolved.

Table 2
Concomitant vaccine antigen GMs 1 month after the infant series and toddler dose (mITT immunogenicity population).

Antigen (Units) Group[a]	After infant series			After toddler dose		
	n[b]	GM[c] (95% CI)[c]	Ratio[c] to Control (95% CI)	n[b]	GM[c] (95% CI)[c]	Ratio[c] to Control (95% CI)
Hib PRP (μg/mL)						
Group 1	136	0.54 (0.44–0.66)	0.93 (0.71–1.22)	126	9.65 (7.74–12.03)	1.08 (0.81–1.43)
Group 3	144	0.49 (0.40–0.60)	0.85 (0.65–1.11)	141	8.25 (6.69–10.16)	0.92 (0.70–1.21)
Group 2	146	0.59 (0.49–0.73)	1.03 (0.79–1.34)	135	9.35 (7.55–11.57)	1.04 (0.79–1.37)
Group 4	139	0.51 (0.42–0.63)	0.89 (0.68–1.16)	138	7.84 (6.35–9.68)	0.87 (0.67–1.16)
Group 5	198	0.58 (0.49–0.69)		202	8.96 (7.53–10.67)	
Pertussis PT (EU/mL)						
Group 1	132	40.86 (36.49–45.76)	0.91 (0.79–1.06)	123	77.43 (68.12–88.02)	1.05 (0.89–1.23)
Group 3	141	40.27 (36.09–44.93)	0.90 (0.78–1.04)	141	73.72 (65.40–83.10)	1.00 (0.85–1.16)
Group 2	143	43.51 (39.02–48.51)	0.97 (0.84–1.12)	137	76.93 (68.13–86.87)	1.04 (0.89–1.22)
Group 4	131	39.26 (35.04–43.98)	0.88 (0.76–1.01)	136	73.38 (64.96–82.90)	0.99 (0.85–1.16)
Group 5	193	44.85 (40.84–49.25)		199	74.01 (66.91–81.86)	
Pertussis FHA (EU/mL)						
Group 1	132	46.29 (41.49–51.65)	9.6 (0.83–1.10)	123	115.55 (104.32–128.00)	0.99 (0.87–1.12)
Group 3	141	41.32 (37.16–45.94)	0.85 (0.74–0.98)	141	123.56 (112.30–135.95)	1.06 (0.93–1.20)
Group 2	143	40.65 (36.59–45.16)	0.84 (0.73–9.6)	137	117.87 (106.98–129.87)	1.01 (0.89–1.14)
Group 4	131	35.55 (31.85–39.68)	0.73 (0.64–0.85)***	136	108.11 (98.09–119.16)	0.92 (0.81–1.05)
Group 5	193	48.42 (44.22–53.01)		199	117.01 (107.97–126.81)	
Pertussis PRN (EU/mL)						
Group 1	132	72.90 (63.26–84.01)	0.86 (0.72–1.04)	123	158.28 (136.30–183.81)	0.92 (0.76–1.11)
Group 3	141	65.82 (57.38–75.50)	0.78 (0.65–0.93)	141	160.96 (139.98–185.08)	0.93 (0.78–1.12)
Group 2	143	71.26 (62.18–81.66)	0.84 (0.70–1.01)	137	156.98 (136.24–180.87)	0.91 (0.76–1.09)
Group 4	131	68.53 (59.44–79.02)	0.81 (0.67–0.97)	136	158.71 (137.67–182.97)	0.92 (0.76–1.10)
Group 5	193	84.57 (75.21–95.09)		199	172.80 (153.64–194.36)	
Tetanus (IU/mL)						
Group 1	132	0.73 (0.65–0.83)	0.90 (0.77–1.06)	123	2.54 (2.28–2.83)	0.96 (0.83–1.10)
Group 3	141	0.69 (0.61–0.77)	0.84 (0.72–0.98)	141	2.60 (2.35–2.88)	0.98 (0.86–1.12)
Group 2	143	0.70 (0.62–0.79)	0.86 (0.74–1.00)	137	2.50 (2.26–2.77)	0.94 (0.82–1.07)
Group 4	131	0.60 (0.53–0.68)	0.74 (0.63–0.87)**	136	2.29 (2.07–2.54)	0.86 (0.75–0.98)
Group 5	193	0.82 (0.74–0.90)		199	2.66 (2.44–2.89)	
Diphtheria (IU/mL)						
Group 1	132	0.62 (0.56–0.69)	0.95 (0.82–1.09)	123	1.64 (1.49–1.80)	0.86 (0.76–0.97)
Group 3	141	0.61 (0.55–0.68)	0.93 (0.81–1.07)	141	1.69 (1.54–1.84)	0.89 (0.79–0.99)
Group 2	143	0.68 (0.61–0.75)	1.03 (0.90–1.19)	137	1.94 (1.77–2.12)	1.02 (0.91–1.14)
Group 4	131	0.65 (0.59–0.73)	1.00 (0.87–1.15)	136	1.87 (1.71–2.04)	0.98 (0.87–1.10)
Group 5	193	0.65 (0.60–0.72)		199	1.90 (1.77–2.05)	
HBV (mIU/mL)						
Group 1	105	756.42 (589.71–970.26)	1.03 (0.75–1.42)	119	4868.61 (3750.57–6319.94)	1.26 (0.90–1.76)
Group 3	120	689.34 (546.12–870.11)	0.94 (0.69–1.28)	133	4250.41 (3320.88–5440.13)	1.10 (0.80–1.52)
Group 2	116	770.93 (608.34–976.98)	1.05 (0.77–1.44)	131	4148.04 (3234.82–5319.08)	1.07 (0.78–1.48)
Group 4	112	599.12 (470.78–762.43)	0.82 (0.60–1.12)	133	4263.28 (3330.93–5456.60)	1.10 (0.80–1.52)
Group 5	156	733.29 (597.81–899.46)		191	3866.37 (3146.78–4750.52)	
Poliomyelitis						
Type 1 (titer)						
Group 1	89	68.11 (53.14–87.30)	0.95 (0.69–1.30)	123	399.56 (332.13–480.68)	0.98 (0.78–1.24)
Group 3	93	67.43 (52.89–85.96)	0.94 (0.68–1.28)	141	443.97 (373.58–527.63)	1.09 (0.87–1.37)
Group 2	105	66.59 (52.98–83.68)	0.92 (0.68–1.25)	133	426.63 (357.15–509.62)	1.05 (0.83–1.32)
Group 4	84	70.66 (54.73–91.23)	0.98 (0.71–1.36)	136	415.45 (348.48–495.29)	1.02 (0.81–1.28)
Group 5	135	72.02 (58.88–88.10)		201	406.37 (351.67–469.59)	
Type 2 (titer)						
Group 1	89	79.60 (61.54–102.95)	1.18 (0.85–1.65)	123	613.18 (515.30–729.65)	0.99 (0.79–1.23)
Group 3	93	62.12 (48.30–79.90)	0.92 (0.66–1.28)	141	587.56 (499.47–691.18)	0.95 (0.77–1.17)
Group 2	105	73.52 (58.01–93.16)	1.09 (0.80–1.50)	133	586.30 (496.01–693.03)	0.94 (0.76–1.17)
Group 4	84	55.17 (42.33–71.89)	0.82 (0.58–1.15)	136	605.78 (513.44–714.73)	0.98 (0.79–1.21)
Group 5	135	67.37 (54.67–83.02)		201	621.07 (542.07–711.57)	
Type 3 (titer)						
Group 1	89	246.22 (192.84–314.38)	1.07 (0.78–1.46)	123	1205.80 (1001.18–1452.24)	0.97 (0.77–1.23)
Group 3	93	257.92 (203.08–327.56)	1.12 (0.82–1.52)	141	1210.29 (1017.32–1439.87)	0.98 (0.78–1.23)
Group 2	105	184.03 (146.96–230.46)	0.80 (0.59–1.08)	133	1045.57 (874.35–1250.32)	0.84 (0.67–1.06)
Group 4	84	218.85 (170.18–281.44)	0.95 (0.69–1.31)	136	1187.11 (994.68–1416.76)	0.96 (0.76–1.21)
Group 5	135	231.02 (189.44–281.72)		201	1237.86 (1070.27–1431.70)	

Note: P values for comparison of the antipyretic groups with control are adjusted using the false discovery rate procedure.
Abbreviations: GM = geometric mean; LSMean = least squares mean; mITT = modified intent-to-treat.
** P < 0.0125.
*** P < 0.001
[a] Group 1, delayed paracetamol; Group 3, concomitant paracetamol; Group 2, delayed ibuprofen; Group 4, concomitant ibuprofen; Group 5, control.
[b] n = number of subjects with a determinate antibody concentration or titer to the given antigen.
[c] GMs were calculated using all subjects with available data for the specified blood draw. CIs are back transformations of CIs based on analysis of log-transformed antibody values using ANOVA with the antipyretic regimen group as a fixed effect. The ratio and related CIs are back transformed from the difference of the LSMean of a specific antipyretic group minus the LSMean of controls.

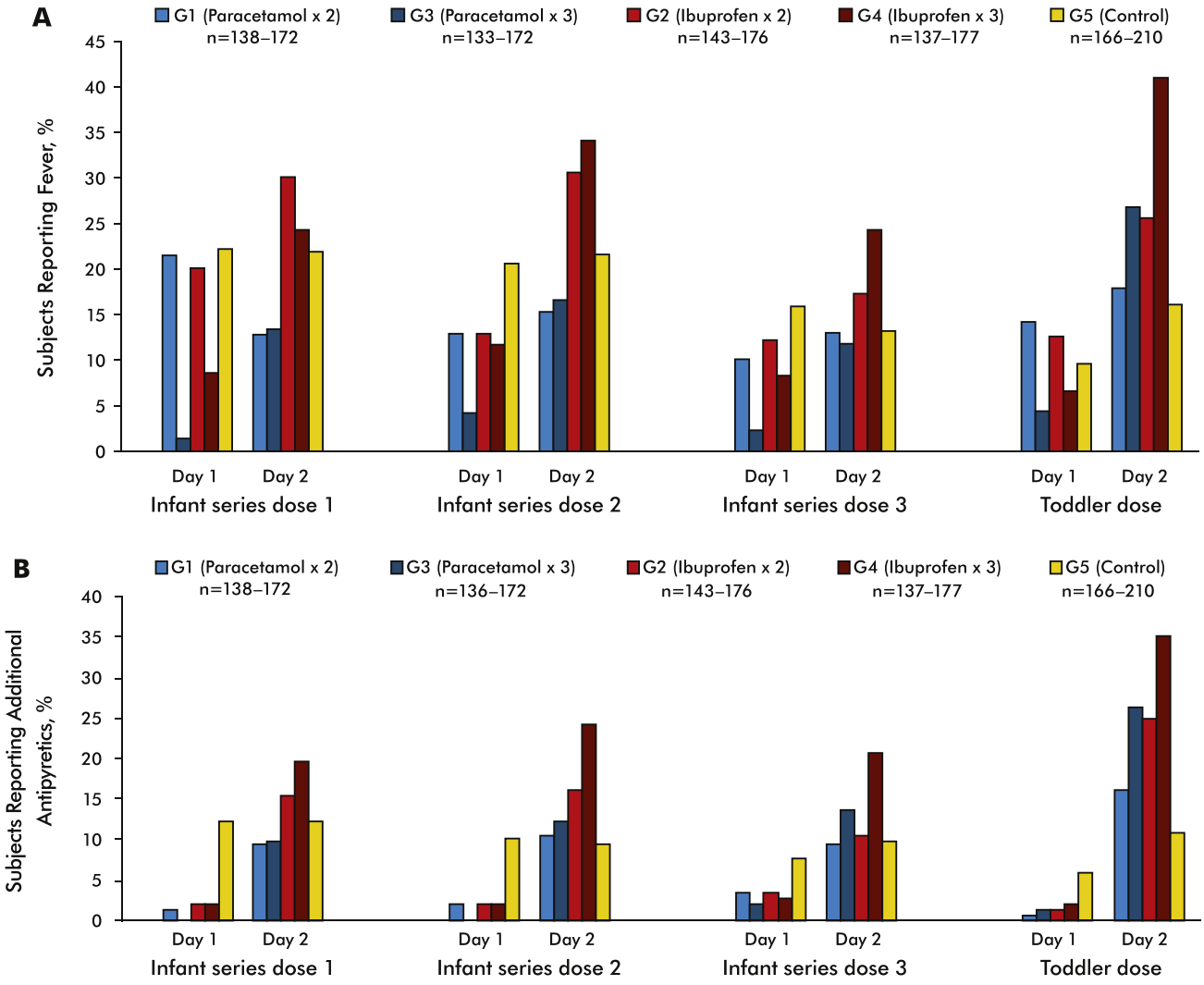

Fig. 2. Incidence of Fever ($\geq$ 38 °C) and Non-Protocol-Specified Antipyretic Use in the First 2 Days After Each Vaccine Dose. (A) Percentage of subjects reporting fever 1 or 2 days postvaccination. (B) Percentage of subjects using non-protocol-specified antipyretic medication in the first 2 days postvaccination. Subject numbers reporting from each group are shown in legends. G1, delayed paracetamol; G3, concomitant paracetamol; G2, delayed ibuprofen; G4, concomitant ibuprofen; G5, control.

4. Discussion

This study is the first randomized controlled trial designed to assess effects of multiple dosing regimens of two commonly used antipyretics on PCV13 and DTaP/HBV/IPV/Hib immunogenicity in a large number of infants and toddlers. These results demonstrate that prophylactic administration of antipyretics to prevent vaccination-associated fever may interfere with immune responses to vaccine antigens. These effects differ by vaccine antigen and antipyretic agent, and may have a total dose- and/or time-dependent administration component.

Changes in pneumococcal immunogenicity were associated with coadministration with paracetamol when given during the primary infant series. While interference was most evident when paracetamol was administered prophylactically concurrent with vaccination, some effects were observed when the first dose was delayed 6–8 h; however, these effects after delayed administration did not achieve statistical significance. In all antipyretic groups, functional antibody levels were not significantly different than in controls, suggesting an acceptable level of protection was elicited in Group 3 despite reduced IgG GMCs for certain serotypes. Additionally, while IgG GMCs were overall lower among subjects receiving paracetamol concurrently with vaccination, this finding reached statistical significance for only some serotypes (serotypes 3, 4, 5, 6B, and 23F). It is not clear whether this response diminution is of clinical significance. Although the percentages of subjects with IgG concentrations $\geq$0.35 μg/mL, the accepted correlate of protection against IPD on a population basis [18], were not substantially different, other benefits of pneumococcal conjugate vaccination that may require higher antibody levels, such as protection against otitis media and reduction of nasopharyngeal colonization leading to indirect effects in the unvaccinated population, may not be fully realized [20].

Ibuprofen had no apparent effect on pneumococcal responses but did appear to affect immune responses to pertussis antigens and tetanus toxoid in DTaP/HBV/IPV/Hib. The role of FHA in the pathogenesis of clinical pertussis disease is unclear. The reduction in response to pertussis FHA after the infant series is of potential concern, given the relatively lower immunity conferred to young infants by acellular pertussis vaccines compared with whole-cell pertussis vaccines [21,22], and the propensity for pertussis FHA immunity to wane over time [23].

This study's findings are generally consistent with those of Prymula et al., in which immune responses were reduced in subjects receiving prophylactic paracetamol compared with controls (though that study evaluated neither ibuprofen nor multiple

antipyretic dosing regimens) [5]. The same group examined effects of paracetamol prophylaxis on immune responses to a meningococcal serogroup B vaccine coadministered with DTaP/HBV/IPV/Hib and PCV7, and found only non-significant reductions in immunogenicity in paracetamol recipients for all 3 vaccines [24]. Those results, however, do not eliminate the possibility of antipyretic interference, as trends in pneumococcal GMC ratios suggested an effect of paracetamol despite satisfactory immune responses [24].

Consistent with previous experience regarding the coadministration of PCV13 and DTaP/HBV/IPV/Hib vaccines, fever was common, self-limiting, and nearly always mild. Expected fever rates on the day of vaccination were lowest for groups receiving antipyretics at vaccination; in subjects receiving delayed antipyretics, fever rates were similar to controls. Paracetamol recipients reported fever less frequently overall than ibuprofen recipients. Additionally, subjects receiving ibuprofen on day 1 experienced more fever on day 2 than other groups, including controls, yielding what may be a "rebound effect" in fever, which to our knowledge has not been described previously. Differences in fever patterns among groups cannot be easily explained, but may be related to characteristics of each antipyretic agent. Although paracetamol and ibuprofen are generally effective and well-tolerated, the risk of adverse reactions and accidental overdose associated with their use in infants and young children [25–29] cannot be discounted when considering the value of fever prevention, especially given that vaccination-associated fever is most often benign and self-limited [1,5,6].

This study was conducted in Poland due to relatively low permissive use of antipyretics [30]. A possible study limitation, however, is the ethnic homogeneity across the subject population. Paracetamol and ibuprofen have variable metabolic properties in different ethnic groups [31–33], suggesting that immune responses in more diverse populations may not follow the trends reported here. Furthermore, larger sample numbers may be required to confirm the emerging trend of lower OPA responses in paracetamol recipients versus controls. Despite a smaller data set, per-protocol infant immunogenicity results were, overall, consistent with those of the mITT and all-available populations, demonstrating that adequate subject numbers were achieved to reach robust conclusions.

The clinical significance of these findings is not known, but suggests that optimal immune response is obtained without prophylactic antipyretics. In animals, ibuprofen has been shown to interfere with the antigen-presenting capability of dendritic cells [34], which play a key role in the development of primary immune responses. Other *in vitro* and *in vivo* studies in human cells and knock-out mice demonstrated that commonly used antipyretic agents may have negative effects on intracellular signaling pathways, cyclooxygenase activity that stimulates prostaglandin release, and on B lymphocytes and antibody production [35–37]. Paracetamol may interfere with leukocyte migration toward the injection site or downstream events such as antigen presentation by dendritic cells. However, the exact mechanism of these effects remains unclear [38]. Such observations have led to concern about antipyretic effects on immune responses to vaccines. Despite our findings, the vaccines studied here continue to be highly effective in populations with high immunization rates, such as those in countries with robust national immunization programs [39–42]. The remarkable effectiveness of pediatric vaccines in such settings suggests that the observable response diminution may have limited clinical relevance on a population basis. However, the potential for reduced immune responses to vaccine antigens should be considered when contemplating the use of prophylactic antipyretics around the time of vaccination. This conclusion is bolstered by other studies that do not support routine prophylactic use of antipyretics in the setting of vaccination, because antipyretics in those studies did not consistently prevent postvaccination fever [1,2,6,43]. Ibuprofen recipients in the current study in fact experienced fever more frequently 2 days after the infant series of vaccinations than those who received no prophylactic antipyretic.

5. Conclusions

The prophylactic use of antipyretics, especially when administered concomitantly with vaccination, may interfere with immune responses to routine vaccines in infants. The effects vary by vaccine, antipyretic agent, and timing of administration. The clinical significance of these findings is unclear, as the immune responses elicited are likely to be sufficient to prevent disease in populations with high immunization rates. Despite the relative reduction in immune responses associated with antipyretic use, priming by the infant series is adequate for a robust response after toddler vaccination. Nonetheless, the data suggest that prophylactic use of over-the-counter antipyretics, especially during the primary infant series of vaccinations, should be considered with caution.

Author Contribution statements

All authors approved and agreed to submit the final article for publication.

Jacek Wysocki: Dr. Wysocki coordinated clinical recruitment of subjects, participated in data acquisition, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Kimberly J. Center: Dr. Center participated in the conceptualization and design of the study, monitored the safety of participating subjects during the study, participated in analysis and interpretation of the data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Jerzy Brzostek: Dr. Brzostek participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Ewa Majda-Stanislawska: Dr. Majda-Stanislawska participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Henryk Szymanski: Dr. Szymanski participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Leszek Szenborn: Dr. Szenborn was involved in recruiting subjects, collecting data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Hanna Czajka: Dr. Czajka participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Barbara Hasiec: Dr. Hasiec participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Jerzy Dziduch: Dr. Dziduch participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Teresa Jackowska: Dr. Jackowska participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Anita Witor: Dr. Witor participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Elżbieta Kopińska: Dr. Kopińska participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Ryszard Konior: Dr. Konior participated in the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Peter C. Giardina: Dr. Giardina supported the acquisition of data and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Vani Sundaraiyer: Dr. Sundaraiyer participated in the statistical analysis, analysis and interpretation of the data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Scott Patterson: Dr. Patterson participated in the conceptualization and design of the study, the statistical analysis, analysis and interpretation of the data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

William C. Gruber: Dr. Gruber supported the conceptualization and design of the study, participated in data analysis, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Daniel A. Scott: Dr. Scott participated in the conceptualization and design of the study, monitored the safety of participating subjects during the study, participated in analysis and interpretation of the data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Alejandra Gurtman: Dr. Gurtman participated in the conceptualization and design of the study, monitored the safety of participating subjects during the study, participated in analysis and interpretation of the data, and contributed to manuscript development, which included review of drafts and approval of the final submitted manuscript.

Funding Statement and role of the sponsor

This study was sponsored by Pfizer Inc. Authors employed by Pfizer were involved in the study design, collection, interpretation, and analysis of data, and in the preparation of the manuscript.

Conflict of interest statement

JW has served as principal investigator in clinical trials sponsored by GlaxoSmithKline, Pfizer, and Novartis and has received grants from the same for participation in conferences. HC has served as a principal investigator in clinical studies sponsored by Pfizer, Novartis, and GlaxoSmithKline. EMS has served as principal investigator in clinical trials sponsored by Pfizer. LS has served as principal investigator in clinical trials sponsored by GlaxoSmithKline, Pfizer, Baxter, and Novartis and has received grants from these vaccine producers and from Sanofi Pasteur for participation in conferences. JB, HS, BH, JD, TJ, AW, EK, and RK have no conflicts of interest to declare. VS is employed by inVentiv Health Clinical, LLC, a company contracted by Pfizer Inc. KJC, PCG, SP, WCG, DAS, and AG are employees of Pfizer Inc.

Acknowledgments

We thank the families who participated in this study, the study investigators, nurses, and coordinators, the clinical testing laboratory staff, and the clinical research associates and scientists at Pfizer Inc. We thank James Trammel, MS, and Gang Sun, MS, MBA, employees of inVentiv Health Clinical, LLC, and paid consultants to Pfizer, for their contributions for the statistical analyses. The authors also thank Margaret Fisher, MD, of Monmouth Medical Center, Drexel University College of Medicine, for her critical review of the manuscript. Dr. Fisher has no conflicts of interest to declare. Medical writing support for all drafts was provided by Jill E. Kolesar, PhD, at Complete Healthcare Communications, LLC, and was funded by Pfizer Inc.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.02.035.

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