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Immunogenicity of pneumococcal conjugate vaccine formulations containing pneumococcal proteins, and immunogenicity and reactogenicity of co-administered routine vaccines – A phase II, randomised, observer-blind study in Gambian infants

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ABSTRACT

Background: Two conserved pneumococcal proteins, pneumolysin toxoid (dPly) and pneumococcal histidine triad protein D (PhtD), combined with 10 polysaccharide conjugates from the pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) in two investigational pneumococcal vaccine (PHiD-CV/dPly/PhtD) formulations were immunogenic and well-tolerated when administered to Gambian children. Here, we report immunogenicity of the polysaccharide conjugates, and immunogenicity and reactogenicity of co-administered routine vaccines.

Methods: In this phase II, controlled, observer-blind, single-centre study, healthy infants aged 8–10 weeks were randomised (1:1:1:1:1:1) to six groups. Four groups received 3+0 schedule (2-3-4 months [M]) of PHiD-CV/dPly/PhtD (10 or 30 μg of each protein), PHiD-CV, or 13-valent pneumococcal conjugate vaccine; and two groups received 2+1 schedule (2-4-9 M) of PHiD-CV/dPly/PhtD (30 μg of each protein) or PHiD-CV. All infants received diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b (DTPw-HBV/Hib) and oral trivalent polio vaccines (OPV) at 2-3-4 M, and measles, yellow fever, and OPV vaccines at 9 M. We evaluated immune responses at 2-5-9-12 M; and reactogenicity 0–3 days post-vaccination.

Results: 1200 infants were enrolled between June 2011 and May 2012; 1152 completed the study. 1 M post-primary vaccination, for each PHiD-CV serotype except 6B and 23F, ≥97.4% (3+0 schedule) and ≥96.4% (2+1 schedule) of infants had antibody concentrations ≥0.2 μg/mL. Immune responses were comparable between groups within the same vaccination schedules. Observed antibody geometric mean concentrations (GMCs) increased by 1 M post-primary vaccination compared to pre-vaccination. In the

Abbreviations: ATP, according-to-protocol; CI, confidence interval; dPly, pneumolysin toxoid; DTPw-HBV/Hib, diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b vaccine; ELISA, enzyme-linked immunosorbent assay; EPI, Expanded Programme on Immunisation; GMC, geometric mean concentration; GMT, geometric mean titre; IPD, invasive pneumococcal disease; IU, International Units; OPA, opsonophagocytic activity; OPV, oral trivalent polio vaccine; PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PHiD-CV, pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine; PHiD-CV/dPly/PhtD, pneumococcal vaccine that contains 10 PHiD-CV polysaccharide conjugates combined with conserved pneumococcal proteins - pneumolysin toxoid and pneumococcal histidine triad protein D; PhtD, pneumococcal histidine triad protein D; PS, polysaccharide; PRP, polyribosyl-ribitol-phosphate; $TCID_{50}$, 50% tissue culture infectious dose; TVC, total vaccinated cohort; WHO, World Health Organization.

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following months, GMCs and opsonophagocytic activity titres waned, with an increase post-booster for the 2+1 schedule. Immune responses to protein D and, DTPw-HBV/Hib, OPV, measles, and yellow fever vaccines were not altered by co-administration with pneumococcal proteins. Reactogenicity of co-administered vaccines was comparable between groups and did not raise concerns.
Conclusion: Immune responses to the 10 PHiD-CV polysaccharide conjugates and co-administered vaccines were not altered by addition of dPly and PhtD. ClinicalTrials.gov identifier NCT01262872.

1. Introduction

Pneumococcal disease mainly affects infants and young children and is responsible for approximately 500,000 deaths of children under 5 years of age every year [1]. While *Streptococcus pneumoniae* has more than 90 serotypes, only 6–11 of these serotypes were responsible for ≥70% of all invasive pneumococcal disease (IPD) globally before the introduction of pneumococcal conjugate vaccines (PCVs) [2,3]. From the year 2000 onwards, PCVs containing capsular polysaccharides (PSs) of the most prevalent IPD-causing serotypes conjugated to a carrier protein have been successfully used in preventing IPD in children worldwide [4–6].

Recently, new formulations containing highly conserved pneumococcal proteins such as pneumolysin toxoid (dPly) and pneumococcal histidine triad protein D (PhtD) have been in development [7,8] and have the potential to offer protection against a wider spectrum of pneumococcal serotypes and prevent serotype emergence and replacement in nasopharyngeal colonisation [8–10]. These new protein antigens were combined with a PCV into one vaccine: the protein-based pneumococcal vaccine containing 10 serotype-specific PS conjugates of the pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV) combined with dPly and PhtD (PHiD-CV/dPly/PhtD) [11–13].

Infant vaccination programmes are becoming increasingly elaborate through the addition of new vaccines that are co-administered to reduce the number of visits [14,15]. Therefore, it was important to assess whether addition of these pneumococcal proteins alter the immune response to the PHiD-CV serotype-specific PS conjugates or to the co-administered routine paediatric vaccines.

This phase II study in Gambian children assessed the efficacy of two formulations of the PHiD-CV/dPly/PhtD vaccine against pneumococcal nasopharyngeal carriage, and their immunogenicity and safety. We previously reported that inclusion of dPly and PhtD in the PHiD-CV/dPly/PhtD investigational formulations had no impact on pneumococcal nasopharyngeal carriage prevalence beyond the protection already provided by the licensed PHiD-CV, regardless of protein dose or schedule; and that the PHiD-CV/dPly/PhtD formulations had an acceptable safety profile in infants [16] and in children 2–4 years of age [17]. Both formulations elicited immune responses to the pneumococcal proteins in infants [16]. Here, we report on the immune response to the serotype-specific PS conjugates, as well as immunogenicity and reactogenicity of co-administered Expanded Programme on Immunisation (EPI) vaccines.

2. Methods

2.1. Study design and participants

This was a phase II, randomised, controlled, observer-blind, single centre study (NCT01262872) conducted in The Gambia between June 2011 and March 2013. Participants were healthy infants aged 8–10 weeks at study start. Inclusion and exclusion criteria were presented previously [16].

Written informed consent was obtained from each parent/legally acceptable representative before vaccination, except for a few deviations as previously presented [16]. The study was conducted in accordance with principles of Good Clinical Practice and the Declaration of Helsinki, and is registered at www.clinicaltrials.gov (NCT01262872).

2.2. Randomisation and blinding

Infants were randomised 1:1:1:1:1:1 into 6 parallel groups to receive PHiD-CV/dPly/PhtD-30, PHiD-CV/dPly/PhtD-10, PHiD-CV (*Synflorix*; GSK), or 13-valent PCV (PCV13; *Prevenar* 13; Pfizer) according to a 3+0 schedule, or PHiD-CV/dPly/PhtD-30 or PHiD-CV in a 2+1 schedule (Fig. 1). We randomly selected 50% of participants (100 per group) for analysis of opsonophagocytic activity (OPA) (OPA subset). In the remaining participants (100 per group), serological testing of co-administered vaccine antigens (co-ad subset) was performed.

Randomisation was performed using MATEX, a block randomisation program developed for use in Statistical Analysis System by GSK. Treatment allocation for pneumococcal vaccines at the investigator site was performed with an internet randomisation system using a minimisation procedure. Treatment numbers were allocated by dose. Co-administered vaccines were managed through sequential numbering of treatment and were administered by the site staff. The study was conducted in an observer-blind manner within each defined pneumococcal vaccination schedule, meaning that vaccine recipients, sponsor, laboratory personnel and anyone responsible for evaluation of any study endpoint were unaware of the administered pneumococcal vaccine.

2.3. Study vaccines

PHiD-CV/dPly/PhtD-10 and PHiD-CV/dPly/PhtD-30 contained the 10 PHiD-CV PS conjugates combined with either 10 or 30 μg of dPly and PhtD each, as detailed previously [17]. PHiD-CV is a suspension of 1 μg of PS for serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3 μg for serotype 4 conjugated to protein D, 3 μg of PS for serotype 18C conjugated to tetanus toxoid, and 3 μg of PS for serotype 19F conjugated to diphtheria toxoid. Its protein carrier content is 9–16 μg of protein D, 3–6 μg of diphtheria toxoid, and 5–10 μg of tetanus toxoid. PCV13 contained 2 μg of each pneumococcal PS for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, and 23F and 4 μg for serotype 6B conjugated to cross-reactive material CRM_{197} carrier protein.

One dose of diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b vaccine (DTPw-HBV/Hib, *Tritanrix*-HepB/Hib, GSK) (0.5 mL) contained ≥30 International Units (IU) of diphtheria toxoid, ≥60 IU of tetanus toxoid, ≥4 IU of killed *Bordetella pertussis*, 10 μg of recombinant hepatitis B surface antigen and a pellet containing 10 μg of polyribosyl-ribitol-phosphate (PRP) conjugated to 20–40 μg of tetanus toxoid to be reconstituted with the DTPw-HBV suspension. One dose of oral trivalent polio vaccine (OPV, *Polio Sabin*, GSK) (0.135 mL) contained a solution with poliovirus type 1 (LS-c, 2ab strain) 10^6 50% tissue culture infectious dose ($TCID_{50}$), poliovirus type 2 (P712, Ch, 2ab strain) 10^5 $TCID_{50}$ and poliovirus type 3 (Leon 12a1b strain) $10^{5.8}$ $TCID_{50}$. Measles vaccine (*M-Vac*, Serum Institute of India) (0.5 mL)

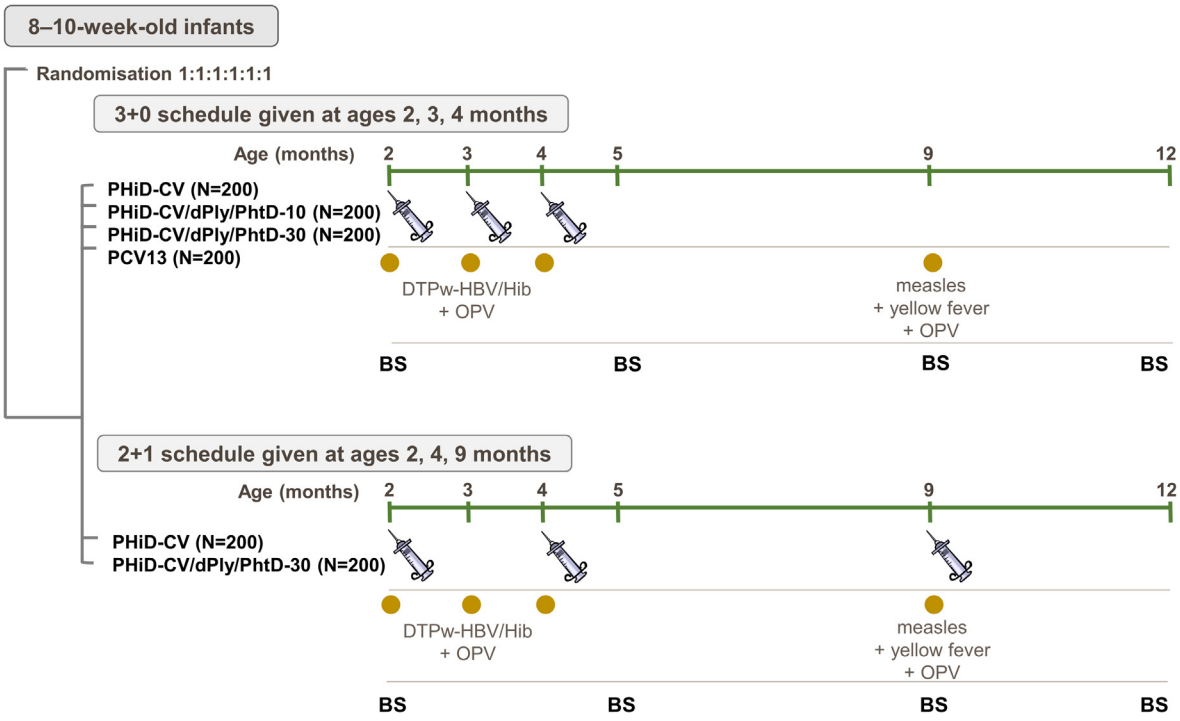

Fig. 1. Study design. PHiD-CV, pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine; PHiD-CV/dPly/PhtD-10, pneumococcal vaccine that contains 10 PHiD-CV polysaccharide conjugates combined with 10 μg pneumolysin toxoid (dPly) and 10 μg pneumococcal histidine triad protein D (PhtD); PHiD-CV/dPly/PhtD-30, pneumococcal vaccine that contains 10 PHiD-CV polysaccharide conjugates combined with 30 μg dPly and 30 μg PhtD; PCV13, 13-valent pneumococcal conjugate vaccine; N, number of infants with available results; , pneumococcal vaccine; , Expanded Programme on Immunisation vaccines; DTPw-HBV/Hib, diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b vaccine; OPV, oral trivalent polio vaccine; BS, blood sample.

contained a freeze-dried pellet of live attenuated measles virus (Edmonston Zagreb strain) ≥1000 50% cell culture infectious dose. Yellow fever vaccine (*Stamaril*, Sanofi Pasteur) (0.5 mL) contained a freeze-dried pellet of live attenuated yellow fever virus (17 D-204 strain) ≥1000 IU.

Pneumococcal vaccines were administered at either 2, 3, and 4 months of age (3+0 schedule) or 2, 4, and 9 months of age (2+1 schedule). All participants received DTPw-HBV/Hib and OPV at 2, 3, and 4 months of age, and measles, yellow fever, and OPV vaccines at 9 months of age (the 4th dose of OPV was added to study procedures to comply with national immunisation programme in The Gambia). Study staff administered pneumococcal vaccines intramuscularly into the right thigh, and co-administered injectable vaccines (DTPw-HBV/Hib, measles, yellow fever) intramuscularly into the left thigh.

2.4. Study objectives

In addition to previously reported objectives [16], study objectives included evaluation of immune responses to the components of the investigational vaccines, other than the pneumococcal protein antigens, and co-administered vaccines, and occurrence of local solicited adverse events at co-administered vaccine injection site (pain, redness, and swelling at injection site) within 4 days (days 0–3) post-each dose. The results of these objectives are disclosed here. The impact of dPly and PhtD in the PHiD-CV/dPly/PhtD investigational formulations on the nasopharyngeal carriage of bacteria other than *Streptococcus pneumoniae* can be found at ClinicalTrials.gov (NCT01262872).

2.5. Immunogenicity assessment

Blood samples were collected from each participant at 2, 5, 9, and 12 months of age (Fig. 1).

Immune responses to pneumococcal vaccines were evaluated pre-vaccination, and 1, 5, and 8 months post-dose 3 for the 3+0 schedule; or pre-vaccination, 1 and 5 months post-dose 2, and 3 months post-dose 3 for the 2+1 schedule. Pneumococcal serotype-specific IgG antibodies were measured by 22F-inhibition enzyme-linked immunosorbent assay (ELISA) for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (assay cut-off: 0.05 μg/mL). Immune responses were described in terms of percentages of infants with IgG concentrations ≥0.2 μg/mL (equivalent to antibody concentrations ≥0.35 μg/mL measured by the non-22F ELISA of the World Health Organization [WHO] reference laboratory) [18]. Pneumococcal serotype-specific OPA was measured at the above-mentioned time points except pre-vaccination, by a killing-assay using an HL60 cell line [19], in single-plex for all serotypes except serotype 19A for which a multiplex assay was used. For all serotypes but 19A, a generic cut-off was applied (opsonic titre of 8), corresponding to the lowest sample dilution in the assays. For 19A, a serotype-specific cut-off was applied, corresponding to the lower limit of quantitation determined for this serotype in the multiplex assay (opsonic titre of 143). Protein D antibodies were quantified using an ELISA with a cut-off of 100 ELISA units (EL.U)/mL.

Immune responses were evaluated 1 month post-dose 3 for the DTPw-HBV/Hib and OPV vaccines, and 3 months after administration of measles and yellow fever vaccines. Diphtheria and tetanus antibody concentrations were measured using standard in-house ELISA; an antibody concentration of ≥0.1 IU/mL was defined as the cut-off for seroprotection. Antibodies against the whole cell *B. pertussis* antigens were determined by ELISA using the IgG enzyme immunoassay test kit from Labsystems (cut-off: 15 EL.U/mL). Antibodies against recombinant hepatitis B surface antigen were measured by an in-house chemiluminescent immunoassay (cut-off: 6.2 mIU/mL). Concentrations of ≥10 mIU/mL were considered protective. Antibody concentrations against the Hib

polysaccharide PRP were measured by ELISA (cut-off: 0.15 μg/mL; this was also the cut-off for seroprotection). Antibody titres ≥8 for poliovirus types 1, 2, and 3 determined by a virus microneutralisation test adapted from WHO guidelines were considered as seropositive and protective [20]. Measles antibodies were titrated using commercially available Enzygnost ELISA kits manufactured by Dade Behring (assay cut-off and cut-off for seroprotection: 150 mIU/mL). Antibodies against yellow fever virus were determined by a plaque reduction neutralisation test by Focus Diagnostics Inc, California, US. Antibody titres ≥10 were considered seroprotective.

2.6. Reactogenicity assessment

Injection site symptoms (pain, redness, and swelling) were collected using diary cards within 4 days post-each dose. Intensity for the local adverse events (AEs) was assessed with grades from 1 to 3; grade 3 for pain was considered crying when limb was moved or limb was spontaneously painful, and for swelling and redness, >30 mm surface diameter. Each parent(s)/legally acceptable representative(s) was instructed to contact the investigator immediately should the infant manifest any signs or symptoms. Trained field workers working under the supervision of the principal investigator visited each vaccinated child on days 1, 2, and 3 following each vaccine dose to collect information on any AEs and to record any medication taken.

2.7. Statistical analysis

Statistical analyses were performed using Statistical Analysis System Discovery Drug on Windows. A target sample size of 170 evaluable participants per group was driven by confirmatory objectives (a sample size of 200 participants per group allowed detection of a 35% reduction in non-PHiD-CV serotypes or serogroups carriage prevalence with 82% power, assuming that non-PHiD-CV serotypes or serogroups carriage prevalence in the comparator PHiD-CV group was 40%; results were previously presented) [16].

Total vaccinated cohort (TVC) for safety included all participants with at least 1 study vaccine administration documented. According-to-protocol (ATP) cohort for immunogenicity analysis included all evaluable participants (i.e. those meeting all eligibility criteria, complying with protocol-defined procedures and intervals, with no elimination criteria during the study) for whom data concerning immunogenicity endpoint measures were available. These included children with results for at least 1 study vaccine antigen component post-vaccination. Maximum interval allowed between primary vaccine doses was 28–48 days for 3+0 schedule and 49–90 days for 2+1 schedule; booster dose was administered at approximately 9–10 months of age.

Geometric mean antibody concentrations (GMCs) and geometric mean titres (GMTs) were calculated with 95% confidence intervals (CIs) by taking the anti-log of the mean of the log antibody concentration or titre transformations. Antibody concentrations and titres below assay cut-offs were given an arbitrary value of half the cut-off for GMC and GMT calculations. Seropositivity/seroprotection rates with exact 95% CIs were calculated for each appropriate serotype/antigen.

3. Results

3.1. Demographic characteristics

Out of 1200 infants in the TVC, 1164 were included in the ATP cohort for immunogenicity and 1152 completed the study. Reasons for withdrawals have been previously published [16]. All infants were African. Demographic characteristics were similar across groups and have been previously published [16].

3.2. Immune response to the 10 PHiD-CV polysaccharide conjugates

Within the same vaccination schedule, the infants' immune responses were similar for the PHiD-CV/dPly/PhtD and PHiD-CV groups in terms of antibody GMCs, OPA GMTs, and proportion of infants with antibody levels and OPA titres above the cut-off values (Tables 1 and 2; Figs. 2 and 3; Tables S1 and S2).

One month post-primary vaccination, for each of the 10 PHiD-CV serotypes common to the pneumococcal study vaccines, ≥97.4% of children in the groups who received a 3+0 schedule and ≥96.4% of children in the groups with a 2+1 schedule had antibody concentrations ≥0.2 μg/mL, except for serotypes 6B (78.4–92.7% [3+0] and 72.0–72.5% [2+1]) and 23F (86.8–97.4% [3+0] and 76.2–77.8% [2+1]) (Table 1). Within the 3+0 schedule, for serotypes 6B and 23F, the percentage of infants with antibody concentrations above the cut-off appeared to be lower in the PHiD-CV group compared to the PCV13 group at 1 month post-primary vaccination; however at 5 and 8 months post-primary vaccination, those percentages for serotypes 6B and 23F appeared lower in the PCV13 group compared to the PHiD-CV group (Table 1). One month post-primary vaccination, for each of the 10 PHiD-CV serotypes, the percentage of children with OPA titres ≥8 was ≥85.3% in the 3+0 groups and ≥82.5% in the 2+1 groups, except for serotype 1 (74.0–90.3% [3+0] and 75.3–75.5% [2+1]) (Table 2).

In both schedules, for all 10 common serotypes, antibody GMCs increased post-primary vaccination but waned in the following months, while remaining above pre-vaccination levels (except for serotype 14 in the 2+1 groups pre-booster vaccination). Within the 3+0 schedule, antibody levels post-primary vaccination appeared lower for serotypes 18C and 19F in the PCV13 group compared with PHiD-CV recipients, while the opposite was observed for serotypes 1, 5, and 14 (Fig. 2 and Table S1). Increases in antibody GMCs were observed post-booster vaccination for PHiD-CV/dPly/PhtD-30 and PHiD-CV administered as a 2+1 schedule. For all PHiD-CV serotypes, observed antibody GMCs at 12 months of age were higher in the 2+1 compared to 3+0 groups in the PHiD-CV-vaccinated groups (i.e. 3 months post-booster for 2+1 or 8 months post-primary vaccination for 3+0 schedules) (Fig. 2 and Table S1). For the majority of the 10 common vaccine pneumococcal serotypes, OPA responses were within similar ranges across all groups at 1 and 5 months post-primary vaccination. Increases in OPA GMTs were observed following booster vaccination in the 2+1 schedule for most serotypes (Fig. 3 and Table S2).

3.3. Immune response to serotypes 3, 6A, and 19A

One month post-primary vaccination, the percentage of infants with antibody concentrations ≥0.2 μg/mL ranged from 6.3% to 10.3% for serotype 3, from 21.9% to 34.6% for serotype 6A and from 47.9% to 56.5% for serotype 19A across all groups except for the PCV13 group, where all infants had antibody concentrations ≥0.2 μg/mL for serotype 3, and 99.5% and 98.4% for serotype 6A and 19A, respectively (Table 1). In the 3+0 and 2+1 schedules, the percentage of infants in the PHiD-CV groups having OPA titres ≥8 ranged from 5.4% to 8.7% for serotype 3 and from 13.4% to 29.5% for serotype 6A. In both schedules, the percentage of infants having 19A OPA titre ≥143 ranged from 35.2% to 59.2%. For the PCV13 group this percentage was 99.0% for serotypes 3 and 6A, and 100% for 19A (Table 2).

In the PCV13 group, observed antibody GMCs for serotypes 3, 6A and 19A increased post-primary vaccination but waned in the following months while remaining above pre-vaccination levels.

Table 1
Percentage of infants with serotype-specific pneumococcal antibody concentrations ≥0.2 μg/mL pre- and post-vaccination (ATP cohort for immunogenicity).

		3+0 schedule								2+1 schedule			
		PHiD-CV/dPly/PhtD-30		PHiD-CV/dPly/PhtD-10		PHiD-CV		PCV13		PHiD-CV/dPly/PhtD-30		PHiD-CV	
Serotype	Time point	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
PHiD-CV vaccine serotypes													
1	M2	195	27.2 (21.1–34.0)	192	30.2 (23.8–37.2)	196	32.7 (26.1–39.7)	194	27.3 (21.2–34.2)	191	35.6 (28.8–42.8)	193	31.1 (24.6–38.1)
	M5	194	100 (98.1–100)	191	100 (98.1–100)	193	100 (98.1–100)	193	100 (98.1–100)	192	100 (98.1–100)	191	100 (98.1–100)
	M9	192	86.5 (80.8–91.0)	190	93.2 (88.6–96.3)	195	90.8 (85.8–94.4)	189	98.9 (96.2–99.9)	189	82.5 (76.4–87.7)	191	86.4 (80.7–90.9)
	M12	189	73.0 (66.1–79.2)	189	76.2 (69.5–82.1)	194	78.4 (71.9–83.9)	187	96.8 (93.1–98.8)	190	97.9 (94.7–99.4)	190	98.4 (95.5–99.7)
4	M2	194	13.9 (9.4–19.6)	192	18.8 (13.5–25.0)	196	14.3 (9.7–20.0)	195	14.4 (9.8–20.1)	193	16.6 (11.6–22.6)	193	20.2 (14.8–26.6)
	M5	195	99.5 (97.2–100)	191	100 (98.1–100)	195	99.5 (97.2–100)	192	100 (98.1–100)	192	97.4 (94.0–99.1)	193	99.0 (96.3–99.9)
	M9	192	100 (98.1–100)	191	99.5 (97.1–100)	194	98.5 (95.5–99.7)	190	97.9 (94.7–99.4)	189	98.9 (96.2–99.9)	190	93.2 (88.6–96.3)
	M12	190	89.5 (84.2–93.5)	189	93.7 (89.2–96.7)	195	91.3 (86.4–94.8)	188	87.8 (82.2–92.1)	190	100 (98.1–100)	190	99.5 (97.1–100)
5	M2	193	22.8 (17.1–29.4)	192	22.9 (17.2–29.5)	193	24.4 (18.5–31.0)	193	20.2 (14.8–26.6)	189	19.0 (13.7–25.4)	192	19.8 (14.4–26.1)
	M5	193	100 (98.1–100)	186	100 (98.0–100)	187	99.5 (97.1–100)	190	100 (98.1–100)	188	100 (98.1–100)	186	98.9 (96.2–99.9)
	M9	192	99.0 (96.3–99.9)	190	98.9 (96.2–99.9)	194	99.0 (96.3–99.9)	190	98.9 (96.2–99.9)	188	98.4 (95.4–99.7)	190	93.7 (89.2–96.7)
	M12	187	89.8 (84.6–93.8)	189	94.2 (89.8–97.1)	194	93.8 (89.4–96.8)	187	96.8 (93.1–98.8)	190	100 (98.1–100)	190	100 (98.1–100)
6B	M2	195	35.4 (28.7–42.5)	190	25.3 (19.3–32.1)	196	29.6 (23.3–36.5)	195	29.7 (23.4–36.7)	192	31.8 (25.3–38.9)	193	34.7 (28.0–41.9)
	M5	194	78.4 (71.9–83.9)	190	85.3 (79.4–90.0)	192	82.3 (76.1–87.4)	191	92.7 (88.0–95.9)	189	72.0 (65.0–78.2)	189	72.5 (65.5–78.7)
	M9	190	90.5 (85.4–94.3)	190	94.2 (89.9–97.1)	194	94.8 (90.7–97.5)	189	77.2 (70.6–83.0)	186	87.1 (81.4–91.6)	189	87.8 (82.3–92.1)
	M12	189	91.5 (86.6–95.1)	189	92.6 (87.9–95.9)	195	94.9 (90.8–97.5)	188	68.1 (60.9–74.7)	190	95.8 (91.9–98.2)	190	96.8 (93.3–98.8)
7F	M2	195	27.7 (21.5–34.5)	192	35.9 (29.2–43.2)	196	29.6 (23.3–36.5)	195	29.7 (23.4–36.7)	193	31.6 (25.1–38.7)	193	31.1 (24.6–38.1)
	M5	194	99.5 (97.2–100)	190	100 (98.1–100)	195	99.0 (96.3–99.9)	195	100 (98.1–100)	192	100 (98.1–100)	191	99.5 (97.1–100)
	M9	192	99.5 (97.1–100)	191	99.5 (97.1–100)	195	100 (98.1–100)	190	100 (98.1–100)	189	99.5 (97.1–100)	191	97.9 (94.7–99.4)
	M12	190	97.9 (94.7–99.4)	189	98.9 (96.2–99.9)	195	99.5 (97.2–100)	188	99.5 (97.1–100)	190	100 (98.1–100)	190	100 (98.1–100)
9V	M2	194	37.1 (30.3–44.3)	192	38.0 (31.1–45.3)	196	38.8 (31.9–46.0)	194	40.2 (33.2–47.5)	193	42.5 (35.4–49.8)	193	31.6 (25.1–38.7)
	M5	194	99.0 (96.3–99.9)	188	100 (98.1–100)	196	98.0 (94.9–99.4)	194	98.5 (95.5–99.7)	191	97.4 (94.0–99.1)	191	98.4 (95.5–99.7)
	M9	192	97.4 (94.0–99.1)	191	97.9 (94.7–99.4)	193	97.4 (94.1–99.2)	189	95.2 (91.2–97.8)	189	96.3 (92.5–98.5)	191	95.3 (91.2–97.8)
	M12	189	95.2 (91.2–97.8)	189	97.9 (94.7–99.4)	195	96.9 (93.4–98.9)	188	88.8 (83.4–93.0)	190	100 (98.1–100)	190	98.9 (96.2–99.9)
14	M2	195	95.9 (92.1–98.2)	192	91.7 (86.8–95.2)	194	95.9 (92.0–98.2)	194	92.3 (87.6–95.6)	192	92.7 (88.1–96.0)	193	94.3 (90.0–97.1)
	M5	193	100 (98.1–100)	191	99.5 (97.1–100)	192	99.5 (97.1–100)	194	100 (98.1–100)	192	99.0 (96.3–99.9)	190	98.9 (96.2–99.9)
	M9	192	95.3 (91.3–97.8)	190	98.4 (95.5–99.7)	194	94.3 (90.1–97.1)	189	94.7 (90.5–97.4)	189	85.2 (79.3–89.9)	191	88.0 (82.5–92.2)
	M12	190	94.7 (90.5–97.4)	189	94.7 (90.5–97.4)	195	93.8 (89.5–96.8)	188	95.2 (91.1–97.8)	190	98.9 (96.2–99.9)	189	98.9 (96.2–99.9)
18C	M2	195	42.1 (35.0–49.3)	192	39.6 (32.6–46.9)	195	41.0 (34.0–48.3)	195	41.0 (34.0–48.3)	193	39.4 (32.4–46.7)	192	40.6 (33.6–47.9)
	M5	194	100 (98.1–100)	186	100 (98.0–100)	189	99.5 (97.1–100)	193	98.4 (95.5–99.7)	191	99.5 (97.1–100)	188	98.9 (96.2–99.9)
	M9	192	100 (98.1–100)	191	100 (98.1–100)	195	99.5 (97.2–100)	190	96.3 (92.6–98.5)	189	96.8 (93.2–98.8)	190	99.5 (97.1–100)
	M12	190	99.5 (97.1–100)	189	100 (98.1–100)	195	99.5 (97.2–100)	188	91.0 (85.9–94.6)	190	100 (98.1–100)	190	100 (98.1–100)
19F	M2	195	77.9 (71.5–83.6)	192	78.6 (72.2–84.2)	195	82.6 (76.5–87.6)	194	82.5 (76.4–87.5)	192	81.8 (75.6–87.0)	192	76.6 (69.9–82.4)
	M5	193	99.0 (96.3–99.9)	189	97.4 (93.9–99.1)	193	97.4 (94.1–99.2)	193	100 (98.1–100)	193	96.4 (92.7–98.5)	192	97.4 (94.0–99.1)
	M9	191	97.9 (94.7–99.4)	190	97.9 (94.7–99.4)	195	97.4 (94.1–99.2)	188	97.9 (94.6–99.4)	187	99.5 (97.1–100)	191	97.4 (94.0–99.1)
	M12	188	94.1 (89.8–97.0)	189	96.3 (92.5–98.5)	194	97.4 (94.1–99.2)	187	82.4 (76.1–87.5)	189	98.9 (96.2–99.9)	189	98.4 (95.4–99.7)
23F	M2	195	36.4 (29.7–43.6)	192	33.9 (27.2–41.0)	196	34.7 (28.1–41.8)	195	34.4 (27.7–41.5)	193	37.8 (31.0–45.1)	193	38.3 (31.5–45.6)
	M5	194	89.2 (83.9–93.2)	185	90.8 (85.7–94.6)	189	86.8 (81.1–91.3)	192	97.4 (94.0–99.1)	189	76.2 (69.5–82.1)	189	77.8 (71.2–83.5)
	M9	192	91.1 (86.2–94.8)	191	89.5 (84.3–93.5)	195	91.3 (86.4–94.8)	190	78.9 (72.5–84.5)	189	78.3 (71.7–84.0)	191	79.1 (72.6–84.6)
	M12	190	84.7 (78.8–89.5)	189	88.4 (82.9–92.6)	195	90.3 (85.2–94.0)	188	67.6 (60.4–74.2)	190	95.3 (91.2–97.8)	190	95.3 (91.2–97.8)
Other serotypes													
3	M2	195	34.4 (27.7–41.5)	192	38.5 (31.6–45.8)	195	31.3 (24.8–38.3)	195	35.4 (28.7–42.5)	193	33.7 (27.1–40.8)	193	33.7 (27.1–40.8)
	M5	192	6.3 (3.3–10.7)	184	10.3 (6.3–15.7)	186	10.2 (6.3–15.5)	192	100 (98.1–100)	186	6.5 (3.4–11.0)	187	9.6 (5.8–14.8)
	M9	192	16.1 (11.2–22.1)	189	15.9 (11.0–21.9)	191	19.4 (14.0–25.7)	188	92.6 (87.8–95.9)	187	13.9 (9.3–19.7)	191	19.9 (14.5–26.3)
	M12	190	26.3 (20.2–33.2)	188	21.8 (16.1–28.4)	195	24.6 (18.7–31.3)	187	67.4 (60.2–74.0)	190	22.1 (16.4–28.7)	190	25.8 (19.7–32.6)

Table 1 (*continued*)

		3+0 schedule							2+1 schedule			
		PHiD-CV/dPly/PhtD-30		PHiD-CV/dPly/PhtD-10		PHiD-CV		PCV13	PHiD-CV/dPly/PhtD-30		PHiD-CV	
Serotype	Time point	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
6A	M2	170	60.6 (52.8–68.0)	163	60.7 (52.8–68.3)	169	63.3 (55.6–70.6)	165	63.0 (55.2–70.4)	171	62.0 (54.3–69.3)	166	62.0 (54.2–69.5)
	M5	192	31.3 (24.8–38.3)	185	34.6 (27.8–41.9)	187	28.9 (22.5–35.9)	190	99.5 (97.1–100)	187	21.9 (16.2–28.5)	187	29.4 (23.0–36.5)
	M9	190	38.4 (31.5–45.7)	190	44.2 (37.0–51.6)	192	51.0 (43.7–58.3)	189	96.8 (93.2–98.8)	185	23.8 (17.8–30.6)	189	30.7 (24.2–37.8)
	M12	179	32.4 (25.6–39.8)	177	40.7 (33.4–48.3)	178	50.0 (42.4–57.6)	148	91.9 (86.3–95.7)	174	36.2 (29.1–43.8)	175	37.1 (30.0–44.8)
19A	M2	193	67.4 (60.3–73.9)	191	68.6 (61.5–75.1)	193	66.3 (59.2–72.9)	193	60.1 (52.8–67.1)	190	62.1 (54.8–69.0)	192	67.7 (60.6–74.3)
	M5	190	47.9 (40.6–55.2)	185	54.1 (46.6–61.4)	182	53.8 (46.3–61.2)	191	98.4 (95.5–99.7)	187	53.5 (46.1–60.8)	186	56.5 (49.0–63.7)
	M9	186	44.6 (37.3–52.1)	187	51.9 (44.5–59.2)	194	57.2 (49.9–64.3)	187	91.4 (86.5–95.0)	183	48.6 (41.2–56.1)	190	45.8 (38.6–53.2)
	M12	185	50.8 (43.4–58.2)	184	54.3 (46.9–61.7)	192	55.2 (47.9–62.4)	185	82.2 (75.9–87.4)	190	68.9 (61.8–75.4)	187	71.7 (64.6–78.0)

Footnote: ATP, according-to-protocol; M, months; N, numbers of infants with available results at each time point; CI, confidence interval; M2, pre-primary vaccination; M5, 1 month post-primary vaccination; M9, 5 months post-primary vaccination (pre-booster in 2+1 groups); M12, 8 months post-primary vaccination in 3+0 groups and 3 months post-booster in 2+1 groups.

In PHiD-CV groups for both schedules, observed antibody GMCs for these 3 serotypes decreased post-primary vaccination, and remained below pre-vaccination levels except for 2+1 groups where an increase in antibody GMCs was observed for serotype 19A post-booster vaccination (Fig. 2 and Table S1). For serotypes 3, 6A and 19A, at each timepoint, OPA responses were within similar ranges across all PHiD-CV groups except an increase in 19A OPA titre post-booster vaccination in the 2+1 groups. Observed OPA GMTs were higher in the PCV13 group and decreased post-primary vaccination (Fig. 3 and Table S2).

3.4. Immune response against protein D

One month post-primary vaccination, in groups receiving the 3+0 schedule, all participants in the PHiD-CV/dPly/PhtD-30, PHiD-CV/dPly/PhtD-10, or PHiD-CV groups and 39.7% of infants in the PCV13 group had anti-protein D concentrations ≥100 EL.U/mL (Table 3), compared to 98.9% and 100% of participants receiving PHiD-CV/dPly/PhtD-30 or PHiD-CV in a 2+1 schedule. Eight months post-primary vaccination, the percentage of children with anti-protein D concentrations ≥100 EL.U/mL had decreased in 3+0 groups, while an increase was reported post-booster for 2+1 groups (Table 3). Increases in anti-protein D GMCs were observed post-vaccination in all groups receiving a protein D-containing vaccine when compared to pre-vaccination levels, regardless of the presence of dPly and PhtD.

3.5. Immune response to the co-administered vaccines

One month post-vaccination, all children in all groups had antibody levels equal to or above the seroprotective threshold for diphtheria, tetanus, and Hib. The observed anti-diphtheria GMC tended to be lower in the PCV13 group compared to the other groups. At least 98.0% of children in each group were considered seroprotected against pertussis, and ≥96.6% were considered seroprotected against hepatitis B (Table 4). For all groups, the percentages of children considered seroprotected against poliovirus 1, 2, and 3 were 89.1–96.8%, 93.4–100%, and 85.3–95.5%, respectively (Table 4). Three months post-vaccination, 72.9–85.6% of children in the 3+0 groups and 82.3–84.0% of children in the 2+1 groups had antibody levels equal to or above the seroprotective level for measles, and 96.9–100% and 95.8–97.9%, respectively, were considered seroprotected against yellow fever (Table 4). In both schedules, immune responses to the co-administered vaccines were similar between PHiD-CV/dPly/PhtD and PHiD-CV groups.

3.6. Reactogenicity of co-administered vaccines

General symptoms, local reactogenicity at the injection site of the pneumococcal vaccines, incidence of unsolicited adverse events after priming and booster vaccine doses, and incidence of serious adverse events were reported previously [16].

For all co-administered vaccine doses, whatever the pneumococcal vaccine and vaccination schedule, injection site pain was the most frequent solicited local symptom both post-primary and post-booster vaccination (Fig. 4). The most frequent grade 3 injection site symptom was swelling, reported in up to 4% of infants in the PHiD-CV/dPly/PhtD-30 (2+1) group following the first dose of the DTPw-HBV/Hib vaccine (Fig. 4).

4. Discussion

Our study evaluated the serotype-specific immune responses to pneumococcal PS conjugates when combined in investigational

Table 2
Percentage of infants with serotype-specific pneumococcal OPA titres above the threshold post-vaccination (ATP cohort for immunogenicity, OPA subset).

		3+0 schedule								2+1 schedule			
		PHiD-CV/dPly/PhtD-30		PHiD-CV/dPly/PhtD-10		PHiD-CV		PCV13		PHiD-CV/dPly/PhtD-30		PHiD-CV	
Serotypes	Time point	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)	N	% (95% CI)
PHiD-CV vaccine serotypes													
1	M5	96	74.0 (64.0–82.4)	93	90.3 (82.4–95.5)	98	88.8 (80.8–94.3)	96	89.6 (81.7–94.9)	98	75.5 (65.8–83.6)	97	75.3 (65.5–83.5)
	M9	95	28.4 (19.6–38.6)	93	38.7 (28.8–49.4)	94	30.9 (21.7–41.2)	90	37.8 (27.8–48.6)	95	23.2 (15.1–32.9)	95	22.1 (14.2–31.8)
	M12	95	25.3 (16.9–35.2)	91	29.7 (20.5–40.2)	96	27.1 (18.5–37.1)	88	35.2 (25.3–46.1)	95	67.4 (57.0–76.6)	95	68.4 (58.1–77.6)
4	M5	95	98.9 (94.3–100)	91	98.9 (94.0–100)	98	100 (96.3–100)	94	100 (96.2–100)	97	100 (96.3–100)	96	95.8 (89.7–98.9)
	M9	91	68.1 (57.5–77.5)	86	84.9 (75.5–91.7)	93	73.1 (62.9–81.8)	84	72.6 (61.8–81.8)	92	63.0 (52.3–72.9)	90	61.1 (50.3–71.2)
	M12	90	56.7 (45.8–67.1)	86	67.4 (56.5–77.2)	91	68.1 (57.5–77.5)	85	58.8 (47.6–69.4)	93	95.7 (89.4–98.8)	91	91.2 (83.4–96.1)
5	M5	95	85.3 (76.5–91.7)	93	95.7 (89.4–98.8)	97	93.8 (87.0–97.7)	96	92.7 (85.6–97.0)	98	86.7 (78.4–92.7)	97	82.5 (73.4–89.4)
	M9	96	53.1 (42.7–63.4)	93	72.0 (61.8–80.9)	97	59.8 (49.3–69.6)	90	64.4 (53.7–74.3)	95	53.7 (43.2–64.0)	95	51.6 (41.1–62.0)
	M12	95	34.7 (25.3–45.2)	92	42.4 (32.1–53.1)	98	49.0 (38.7–59.3)	89	50.6 (39.8–61.3)	96	91.7 (84.2–96.3)	95	85.3 (76.5–91.7)
6B	M5	95	89.5 (81.5–94.8)	93	95.7 (89.4–98.8)	96	95.8 (89.7–98.9)	96	94.8 (88.3–98.3)	95	87.4 (79.0–93.3)	96	87.5 (79.2–93.4)
	M9	91	87.9 (79.4–93.8)	93	89.2 (81.1–94.7)	97	92.8 (85.7–97.0)	88	64.8 (53.9–74.7)	91	80.2 (70.6–87.8)	91	73.6 (63.3–82.3)
	M12	88	80.7 (70.9–88.3)	90	83.3 (74.0–90.4)	97	90.7 (83.1–95.7)	83	69.9 (58.8–79.5)	94	89.4 (81.3–94.8)	89	89.9 (81.7–95.3)
7F	M5	95	100 (96.2–100)	93	100 (96.1–100)	98	99.0 (94.4–100)	96	100 (96.2–100)	98	100 (96.3–100)	96	100 (96.2–100)
	M9	96	100 (96.2–100)	93	100 (96.1–100)	97	99.0 (94.4–100)	90	100 (96.0–100)	95	98.9 (94.3–100)	94	100 (96.2–100)
	M12	95	100 (96.2–100)	92	100 (96.1–100)	98	100 (96.3–100)	89	100 (95.9–100)	96	100 (96.2–100)	95	100 (96.2–100)
9V	M5	96	99.0 (94.3–100)	93	100 (96.1–100)	98	96.9 (91.3–99.4)	94	96.8 (91.0–99.3)	98	99.0 (94.4–100)	96	100 (96.2–100)
	M9	95	98.9 (94.3–100)	92	95.7 (89.2–98.8)	97	95.9 (89.8–98.9)	90	94.4 (87.5–98.2)	91	89.0 (80.7–94.6)	94	87.2 (78.8–93.2)
	M12	92	95.7 (89.2–98.8)	89	95.5 (88.9–98.8)	97	90.7 (83.1–95.7)	88	94.3 (87.2–98.1)	95	100 (96.2–100)	95	96.8 (91.0–99.3)
14	M5	95	97.9 (92.6–99.7)	93	95.7 (89.4–98.8)	97	95.9 (89.8–98.9)	93	96.8 (90.9–99.3)	98	90.8 (83.3–95.7)	96	87.5 (79.2–93.4)
	M9	95	87.4 (79.0–93.3)	89	92.1 (84.5–96.8)	95	89.5 (81.5–94.8)	89	94.4 (87.4–98.2)	89	70.8 (60.2–79.9)	91	65.9 (55.3–75.5)
	M12	89	87.6 (79.0–93.7)	88	86.4 (77.4–92.8)	95	89.5 (81.5–94.8)	89	93.3 (85.9–97.5)	93	100 (96.1–100)	93	93.5 (86.5–97.6)
18C	M5	96	97.9 (92.7–99.7)	93	100 (96.1–100)	98	98.0 (92.8–99.8)	94	97.9 (92.5–99.7)	98	90.8 (83.3–95.7)	97	94.8 (88.4–98.3)
	M9	95	75.8 (65.9–84.0)	92	83.7 (74.5–90.6)	96	85.4 (76.7–91.8)	90	37.8 (27.8–48.6)	93	72.0 (61.8–80.9)	95	76.8 (67.1–84.9)
	M12	92	52.2 (41.5–62.7)	88	52.3 (41.4–63.0)	97	61.9 (51.4–71.5)	88	21.6 (13.5–31.6)	96	97.9 (92.7–99.7)	94	98.9 (94.2–100)
19F	M5	95	92.6 (85.4–97.0)	90	95.6 (89.0–98.8)	98	96.9 (91.3–99.4)	94	94.7 (88.0–98.3)	96	92.7 (85.6–97.0)	96	92.7 (85.6–97.0)
	M9	96	76.0 (66.3–84.2)	92	85.9 (77.0–92.3)	98	89.8 (82.0–95.0)	91	22.0 (14.0–31.9)	93	76.3 (66.4–84.5)	94	76.6 (66.7–84.7)
	M12	94	58.5 (47.9–68.6)	92	67.4 (56.8–76.8)	97	73.2 (63.2–81.7)	89	19.1 (11.5–28.8)	96	89.6 (81.7–94.9)	95	85.3 (76.5–91.7)
23F	M5	94	89.4 (81.3–94.8)	93	93.5 (86.5–97.6)	98	92.9 (85.8–97.1)	96	94.8 (88.3–98.3)	94	86.2 (77.5–92.4)	95	85.3 (76.5–91.7)
	M9	87	85.1 (75.8–91.8)	85	82.4 (72.6–89.8)	90	76.7 (66.6–84.9)	86	83.7 (74.2–90.8)	90	70.0 (59.4–79.2)	91	70.3 (59.8–79.5)
	M12	81	82.7 (72.7–90.2)	87	79.3 (69.3–87.3)	89	83.1 (73.7–90.2)	83	83.1 (73.3–90.5)	95	91.6 (84.1–96.3)	91	91.2 (83.4–96.1)
Other serotypes													
3	M5	92	5.4 (1.8–12.2)	92	8.7 (3.8–16.4)	97	7.2 (3.0–14.3)	96	99.0 (94.3–100)	96	8.3 (3.7–15.8)	97	7.2 (3.0–14.3)
	M9	94	12.8 (6.8–21.2)	91	22.0 (14.0–31.9)	94	20.2 (12.6–29.8)	89	58.4 (47.5–68.8)	87	17.2 (10.0–26.8)	86	14.0 (7.4–23.1)
	M12	91	28.6 (19.6–39.0)	86	29.1 (19.8–39.9)	95	26.3 (17.8–36.4)	87	43.7 (33.1–54.7)	92	22.8 (14.7–32.8)	93	23.7 (15.5–33.6)
6A	M5	91	27.5 (18.6–37.8)	88	29.5 (20.3–40.2)	96	26.0 (17.6–36.0)	96	99.0 (94.3–100)	96	14.6 (8.2–23.3)	97	13.4 (7.3–21.8)
	M9	92	25.0 (16.6–35.1)	92	30.4 (21.3–40.9)	94	37.2 (27.5–47.8)	88	94.3 (87.2–98.1)	93	28.0 (19.1–38.2)	95	25.3 (16.9–35.2)
	M12	86	25.6 (16.8–36.1)	86	29.1 (19.8–39.9)	88	36.4 (26.4–47.3)	87	83.9 (74.5–90.9)	88	26.1 (17.3–36.6)	91	18.7 (11.3–28.2)
19A	M5	72	45.8 (34.0–58.0)	70	54.3 (41.9–66.3)	71	59.2 (46.8–70.7)	89	100 (95.9–100)	69	43.5 (31.6–56.0)	71	35.2 (24.2–47.5)
	M9	75	21.3 (12.7–32.3)	71	19.7 (11.2–30.9)	61	26.2 (15.8–39.1)	69	79.7 (68.3–88.4)	67	13.4 (6.3–24.0)	81	13.6 (7.0–23.0)
	M12	79	22.8 (14.1–33.6)	67	25.4 (15.5–37.5)	69	23.2 (13.9–34.9)	60	58.3 (44.9–70.9)	72	44.4 (32.7–56.6)	79	53.2 (41.6–64.5)

Footnote: OPA, opsonophagocytic activity; ATP, according-to-protocol; CI, confidence interval; M, months; N, numbers of infants with available results at each time point; M5, 1 month post-primary vaccination; M9, 5 months post-primary vaccination (pre-booster in 2+1 groups); M12, 8 months post-primary vaccination in 3+0 groups and 3 months post-booster in 2+1 groups. The OPA titre threshold is 8 for all serotypes except for 19A, for which the serotype-specific threshold is 143.

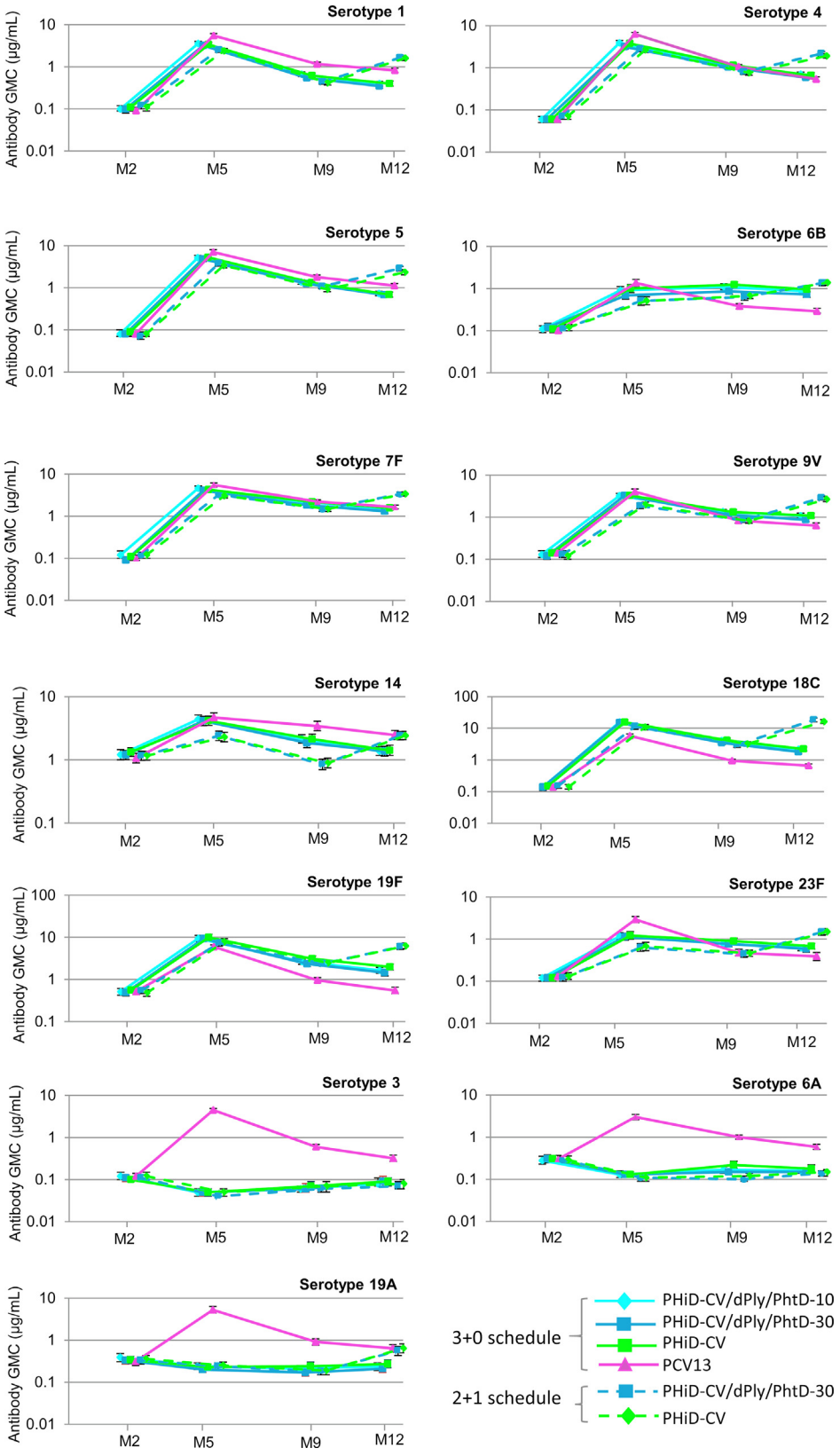

Fig. 2. Kinetics of serotype-specific pneumococcal antibody GMCs (ATP cohort for immunogenicity). ATP, according-to-protocol; GMC, geometric mean concentration; M, months; M2, pre-primary vaccination; M5, 1 month post-primary vaccination; M9, 5 months post-primary vaccination (pre-booster in 2+1 groups); M12, 8 months post-primary vaccination in 3+0 groups and 3 months post-booster in 2+1 groups. Note: Error bars indicate 95% confidence intervals. Data for the groups are slightly shifted for better visualisation.

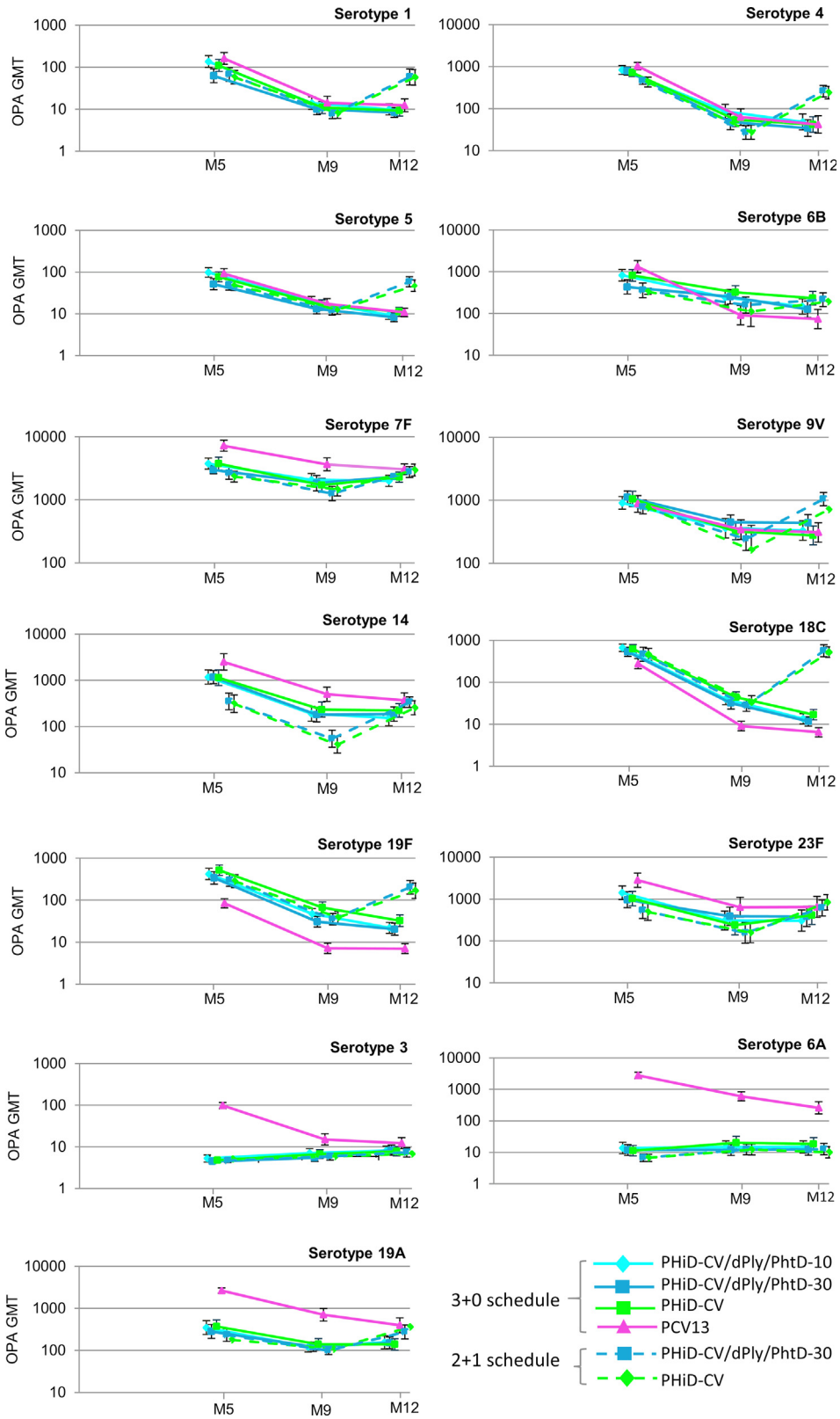

Fig. 3. Kinetics of serotype-specific pneumococcal OPA GMTs titres (ATP cohort for immunogenicity, OPA subset). ATP, according to protocol; GMT, geometric mean titre; M, months; M5, 1 month post-primary vaccination; M9, 5 months post-primary vaccination (pre-booster in 2+1 groups); M12, 8 months post-primary vaccination in 3+0 groups and 3 months post-booster in 2+1 groups; OPA, opsonophagocytic activity. Note: Error bars indicate 95% confidence intervals. Data for the groups are slightly shifted for better visualisation.

formulations with pneumococcal proteins dPly and PhtD, as well as the immune response and reactogenicity to the co-administered EPI vaccines.

Both PHiD-CV/dPly/PhtD-10 and PHiD-CV/dPly/PhtD-30 induced immune responses against each of the PHiD-CV vaccine serotypes and against protein D, in similar ranges as observed in

PHiD-CV vaccinees. These results are in line with previous findings from European studies assessing pneumococcal protein-containing formulations in infants [13] and toddlers [12]. Efficacy/effectiveness data of PHiD-CV have been reported for various clinical trials and post-marketing surveillance studies [21–24]. Based on the observed immune responses to PHiD-CV in the current study, showing no substantial difference when combined in PHiD-CV/dPly/PhtD formulations, we can presume that the vaccine efficacy of the PS conjugates against pneumococcal disease would remain unaffected when combined with dPly and PhtD.

Pneumococcal protein antigens seemed not to alter immune responses to co-administered EPI vaccines (DTPw-HBV/Hib, OPV, measles, and yellow fever). Similarly, in a study in European children, the immune response induced by DTPa-HBV-IPV/Hib vaccine did not appear altered when co-administered at 2, 3, 4, and 12–15 months of age with PHiD-CV/dPly/PhtD-10 or PHiD-CV/dPly/PhtD-30 formulations [13].

No apparent differences in reactogenicity were observed when co-administering DTPw-HBV/Hib, measles and yellow fever vaccines with PHiD-CV/dPly/PhtD formulations compared to co-administration with PHiD-CV. Pain was the most commonly reported injection site symptom for co-administered vaccines, in line with results described by Dicko et al. [25] in children in the same age group in African settings who received PHiD-CV co-administered with DTPw-HBV/Hib. Incidences of pain and swelling at the DTPw-HBV/Hib injection site were higher in the study of Dicko et al. [25] than observed in the current study. This disparity could be due to the different DTPw-HBV/Hib vaccines that were administered (*Zilbrix* in the previous study [25], *Tritanrix*-HepB/Hib in this study), or to differences in the method used in the reactogenicity data collection. Reactogenicity data were collected by field workers in our study, but by either field workers (in Nigeria) or study physicians (in Mali) in the study by Dicko et al [25]. Another study with PHiD-CV and DTPw-HBV/Hib (*Tritanrix*-HepB/Hib) co-administration in an African setting also reported pain as the most frequent injection site symptom for observations combined for PHiD-CV and DTPw-HBV/Hib injection sites, but injection site swelling was rare and no redness or grade 3 injection site symptoms were reported [26]. These results were similar to our study where low percentages of infants with injection site swelling and redness were reported.

As this study used both PHiD-CV and PCV13 as controls, it provided a unique opportunity to assess their immunogenicity in this setting. It has to be noted, however, that our study was not designed for such inter-group comparisons and these findings should be interpreted with caution, especially considering that many factors could play a role (e.g. size and quantity of the PSs, protein carriers and conjugation methods, manufacturing process) and influence antibody functionality. For the majority of the common serotypes, no major differences were observed between PHiD-CV and PCV13. Immune responses for serotypes 18C and 19F tended to be higher for the PHiD-CV group compared with the PCV13 group while immune responses for serotypes 1, 5, and 14 appeared higher for the PCV13 group. For serotypes 6B and 23F, at 1 month post-primary vaccination, immune responses appeared to be lower in the PHiD-CV group compared to the PCV13 group; however, this trend reversed at later time points, suggesting a difference in antibody kinetics and persistence for these serotypes elicited by the two vaccines. In the non-inferiority study for PHiD-CV licensure, non-inferiority of PHiD-CV compared to PCV7 could not be shown for serotypes 6B and 23F at 1 month post-primary vaccination [27]. However, several trials and post-marketing studies have reported efficacy data for PHiD-CV [21–24], and efficacy/effectiveness of both PHiD-CV and PCV13 is further exemplified by a recent review paper [28]. A systematic review of literature on the impact or effectiveness of PHiD-CV and PCV13 on deaths or

Table 3
Percentage of infants with anti-protein D antibody concentrations ≥100 ELU/mL and antibody GMCs (ATP cohort for immunogenicity).

Time point	3+0 schedule										2+1 schedule					
	PHiD-CV/dPly/PhtD-30			PHiD-CV/dPly/PhtD-10			PHiD-CV			PCV13			PHiD-CV/dPly/PhtD-30			PHiD-CV		
	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMC (95% CI)	N	% (95% CI)	GMC (95% CI)
M2	192	17.7 (12.6–23.9)	70.9 (62.8–80.0)	187	17.1 (12.0–23.3)	65.1 (59.4–71.5)	196	22.4 (16.8–28.9)	75.5 (67.0–85.1)	194	19.6 (14.2–25.9)	71.9 (64.0–80.7)	192	19.3 (13.9–25.6)	72.1 (63.8–81.4)	190	13.2 (8.7–18.8)	62.5 (57.1–68.4)
M5	193	100 (98.1–100)	1833.8 (1628.5–2065.0)	186	100 (98.0–100)	1922.8 (1739.3–2125.6)	187	100 (98.0–100)	1990.2 (1765.7–2243.3)	189	39.7 (32.7–47.0)	84.8 (76.4–94.1)	187	98.9 (96.2–99.9)	990.9 (869.3–1129.5)	187	100 (98.0–100)	1126.7 (999.4–1270.2)
M9	191	95.8 (91.9–98.2)	499.2 (439.9–566.5)	191	97.4 (94.0–99.1)	559.2 (496.8–629.6)	193	98.4 (95.5–99.7)	609.9 (537.0–692.8)	185	38.9 (31.9–46.3)	83.1 (74.9–92.2)	188	89.9 (84.7–93.8)	281.3 (247.5–319.7)	187	92.0 (87.1–95.4)	313.0 (276.2–354.7)
M12	190	86.8 (81.2–91.3)	288.2 (248.5–334.2)	187	90.9 (85.8–94.6)	324.3 (282.2–372.7)	194	92.3 (87.6–95.6)	344.2 (300.6–394.2)	187	26.7 (20.5–33.7)	69.2 (63.6–75.3)	190	97.9 (94.7–99.4)	534.8 (468.9–610.0)	190	96.8 (93.3–98.8)	664.9 (580.1–762.2)

Footnote: ELU/mL, ELISA (enzyme linked immunosorbent assay) units per millilitre; ATP, according-to-protocol; CI, confidence interval; GMC, geometric mean concentration; M, months; N, numbers of infants with available results at each time point; M2, pre-primary vaccination; M5, 1 month post-primary vaccination (pre-booster in 2+1 groups); M12, 8 months post-primary vaccination in 3+0 groups and 3 months post-booster in 2+1 groups.

Table 4
Immune response to co-administered DTPw-HBV/Hib, OPV, measles, and yellow fever vaccines (ATP cohort for immunogenicity, co-ad subset).

Antibody (Cut-off) Time point		3+0 schedule								2+1 schedule			
		N	PHiD-CV/dPly/PhtD-30	N	PHiD-CV/dPly/PhtD-10	N	PHiD-CV	N	PCV13	N	PHiD-CV/dPly/PhtD-30	N	PHiD-CV
Anti-D (≥0.1 IU/mL) M5	% (95% CI) GMC (95% CI)	99	100 (96.3–100) 2.7 (2.4–3.1)	99	100 (96.3–100) 2.5 (2.1–3.0)	98	100 (96.3–100) 2.9 (2.5–3.3)	99	100 (96.3–100) 1.5 (1.3–1.8)	95	100 (96.2–100) 2.6 (2.2–3.0)	96	100 (96.2–100) 2.6 (2.2–3.0)
Anti-T (≥0.1 IU/mL) M5	% (95% CI) GMC (95% CI)	99	100 (96.3–100) 5.1 (4.3–6.1)	99	100 (96.3–100) 5.0 (4.1–6.0)	98	100 (96.3–100) 4.7 (4.0–5.6)	99	100 (96.3–100) 4.0 (3.5–4.7)	95	100 (96.2–100) 5.7 (4.8–6.8)	96	100 (96.2–100) 4.7 (3.9–5.7)
Anti-BP (≥15 EL.U/mL) M5	% (95% CI) GMC (95% CI)	99	100 (96.3–100) 110.3 (99.3–122.5)	99	99.0 (94.5–100) 111.9 (99.4–125.8)	98	98.0 (92.8–99.8) 105.8 (94.4–118.6)	99	99.0 (94.5–100) 117.0 (105.0–130.3)	94	100 (96.2–100) 123.2 (112.0–135.5)	96	99.0 (94.3–100) 114.7 (101.3–129.9)
Anti-PRP (≥0.15 µg/mL) M5	% (95% CI) GMC (95% CI)	99	100 (96.3–100) 19.4 (15.3–24.6)	99	100 (96.3–100) 23.4 (19.3–28.3)	98	100 (96.3–100) 19.2 (15.1–24.5)	99	100 (96.3–100) 19.0 (15.2–23.6)	95	100 (96.2–100) 21.3 (17.9–25.3)	96	100 (96.2–100) 21.2 (17.4–25.7)
Anti-HBs (≥10 mIU/mL) M5	% (95% CI) GMC (95% CI)	93	100 (96.1–100) 1165.8 (910.7–1492.3)	94	98.9 (94.2–100) 1235.8 (946.9–1612.9)	91	97.8 (92.3–99.7) 990.1 (757.6–1294.0)	89	98.9 (93.9–100) 1206.6 (946.2–1538.7)	89	98.9 (93.9–100) 1318.5 (1062.5–1636.3)	88	96.6 (90.4–99.3) 976.5 (724.2–1316.8)
Anti-Polio 1 (≥8) M5	% (95% CI) GMT (95% CI)	92	89.1 (80.9–94.7) 314.8 (202.7–488.7)	97	92.8 (85.7–97.0) 413.2 (287.0–594.9)	91	91.2 (83.4–96.1) 447.9 (278.5–720.1)	94	90.4 (82.6–95.5) 398.3 (256.6–618.3)	87	93.1 (85.6–97.4) 330.3 (208.6–523.0)	93	96.8 (90.9–99.3) 415.6 (286.7–602.6)
Anti-Polio 2 (≥8) M5	% (95% CI) GMT (95% CI)	78	100 (95.4–100) 619.5 (462.0–830.7)	76	93.4 (85.3–97.8) 545.8 (371.6–801.6)	76	96.1 (88.9–99.2) 514.2 (353.8–747.2)	73	97.3 (90.5–99.7) 536.9 (400.6–719.6)	75	94.7 (86.9–98.5) 486.7 (319.7–741.1)	82	100 (95.6–100) 702.9 (529.6–932.8)
Anti-Polio 3 (≥8) M5	% (95% CI) GMT (95% CI)	88	95.5 (88.8–98.7) 166.0 (124.5–221.5)	80	86.3 (76.7–92.9) 135.4 (91.5–200.5)	82	85.4 (75.8–92.2) 110.1 (72.5–167.2)	84	88.1 (79.2–94.1) 191.8 (129.4–284.4)	75	85.3 (75.3–92.4) 106.9 (71.8–159.1)	80	93.8 (86.0–97.9) 181.1 (131.7–249.0)
Anti-Measles (≥150 mIU/mL) M12	% (95% CI) GMC (95% CI)	95	85.3 (76.5–91.7) 298.6 (254.9–349.8)	90	85.6 (76.6–92.1) 329.2 (272.6–397.5)	96	72.9 (62.9–81.5) 295.8 (237.2–368.8)	86	77.9 (67.7–86.1) 274.1 (227.7–330.0)	96	82.3 (73.2–89.3) 284.5 (240.9–336.0)	94	84.0 (75.0–90.8) 305.9 (256.5–364.8)
Anti-YFV (≥10) M12	% (95% CI) GMT (95% CI)	95	98.9 (94.3–100) 264.7 (201.0–348.5)	97	96.9 (91.2–99.4) 334.0 (250.6–445.1)	97	100 (96.3–100) 379.9 (293.2–492.2)	98	98.0 (92.8–99.8) 306.7 (242.9–387.3)	94	97.9 (92.5–99.7) 342.0 (252.2–463.7)	95	95.8 (89.6–98.8) 239.0 (176.0–324.5)

Footnote: DTPw-HBV/Hib, diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b vaccine; OPV, oral trivalent polio vaccine; ATP, according-to-protocol; N, numbers of infants with available results; M, months; Anti-D, anti-diphtheria; Anti-T, anti-tetanus; Anti-BP, anti-*Bordetella pertussis*; Anti-PRP, anti-polyribosyl-ribitol-phosphate; anti-HBs, anti-hepatitis B; anti-YFV, anti-yellow fever; IU/mL, international units per millilitre; EL.U/mL, ELISA (enzyme linked immunosorbent assay) units per millilitre; M5, 1 month post-primary vaccination; M12, 3 months post-vaccination; CI, confidence interval; GMC, geometric mean concentration; GMT, geometric mean titre.

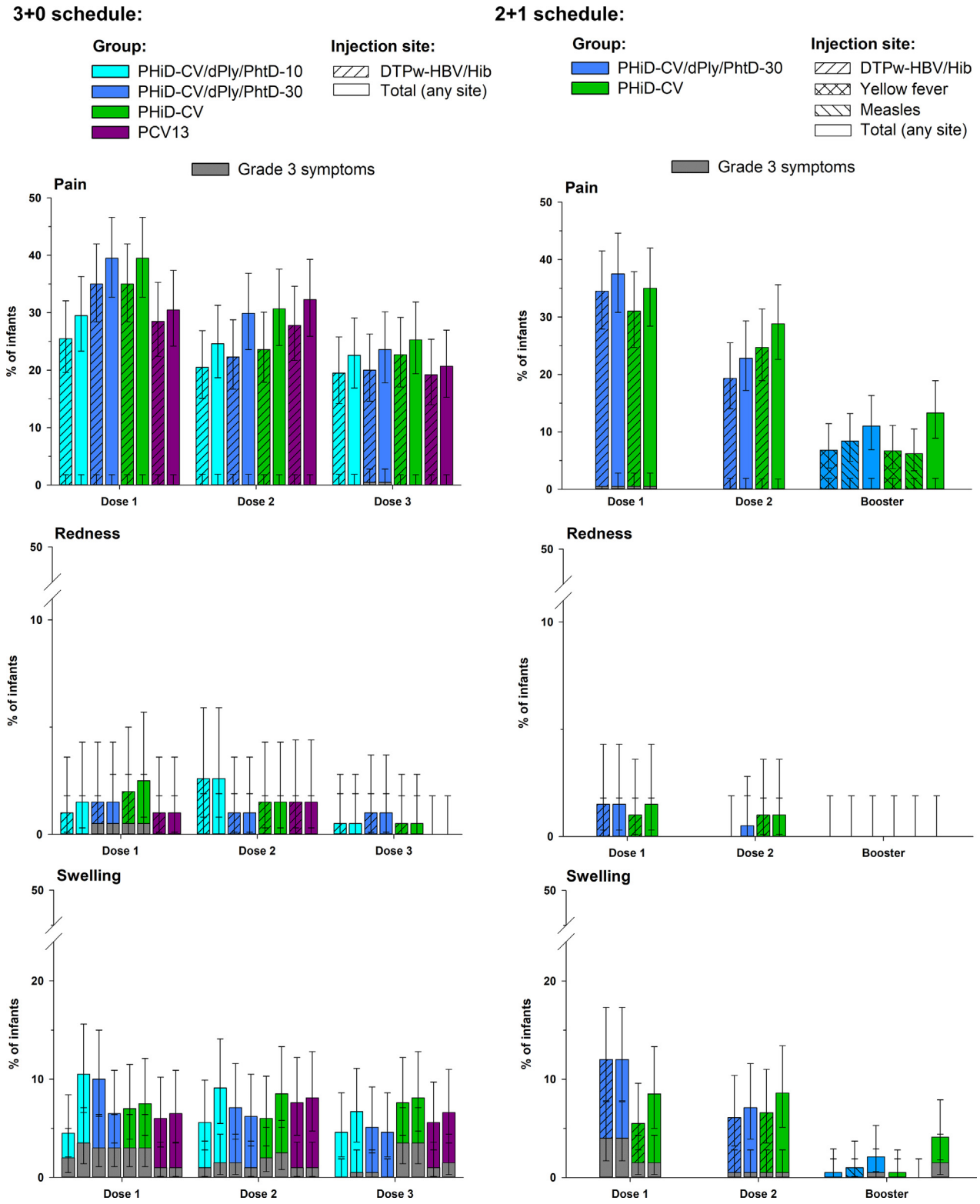

Fig. 4. Percentage of infants reporting at least 1 solicited local symptom, at injection site and overall at any of the vaccine injection sites, for EPI routine vaccines co-administered with pneumococcal vaccines (total vaccinated cohort). EPI, Expanded Programme on Immunisation; In the 3+0 schedule, diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b vaccine (DTPw-HBV/Hib) and oral trivalent polio (OPV) vaccines were co-administered with pneumococcal vaccines at 2, 3, 4, months of age. In the 2+1 schedule, DTPw-HBV/Hib and OPV were co-administered with pneumococcal vaccines at 2, 4 months of age and measles, yellow fever, and OPV vaccines at 9 months of age. Grade 3 for pain was considered crying when limb was moved or limb was spontaneously painful, and for swelling and redness, >30 mm surface diameter. Note: Error bars indicate 95% confidence intervals.

hospitalisation due to IPD, pneumonia, meningitis, and sepsis in children below 5 years of age, also showed that there was no evidence of superiority of one vaccine over the other [6].

A limitation of this study is the fact that the assessment of immune responses for the PS conjugates and the co-administered vaccines was descriptive, which was justified by the phase II design

of the study. Also, due to the non-standard interval used for post-booster timepoint (3 months from last dose), no direct comparison between post-primary (1 month from last dose) and post-booster vaccinations should be made. Another limitation is the fact that polio vaccination campaigns were run in The Gambia in 2011 and 2012. Some of the children may have received additional doses of polio vaccine; these children were not eliminated from the ATP cohort for immunogenicity. Thus, results for those antigens should be interpreted with caution as they may not reflect only study vaccination. Reactogenicity assessment for co-administered vaccines was performed only after vaccination visits where pneumococcal vaccines were administered, e.g. it was not done for measles and yellow fever vaccines given at 10 months of age in 3+0 groups, and post-dose 2 of DTPw-HBV/Hib vaccine for the 2+1 groups since no pneumococcal vaccine was co-administered.

To conclude, both pneumococcal vaccine formulations containing the proteins dPly and PhtD induced similar immune responses against the 10 common vaccine serotypes and protein D at all post-vaccination time points as observed for PHiD-CV. No differences were observed in the immune responses to DTPw-HBV/Hib, OPV, measles and yellow fever vaccines used for infant EPI vaccinations in The Gambia, and reported reactogenicity rates for co-administered vaccines were also comparable between groups.

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Data sharing statement

A protocol summary and the results summary for this study (GSK study number 114174 – NCT01262872) are available on the GSK Clinical Study Register and can be accessed at https://www.gsk-studyregister.com. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

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Authors' contributions

AO was involved in planning, data collection, site coordination of study, review of the reported study, and trained and supervised clinicians and staff on study procedures, clinically evaluated and investigated the patients, maintained quality assurance over clinical procedures, and drafted the report. MOCO was involved in planning, data collection, review, project oversight on site and was involved in the conception of the study design, trained and supervised clinicians and staff on study procedures, clinically evaluated and investigated the patients, maintained quality assurance over clinical procedures, and supervised the laboratory work. MAn was involved in planning, designing, and reviewing the reported study, the analysis plan, and interpretation of the results. EOO clinically evaluated and investigated the patients and maintained quality assurance over clinical procedures. EOO also participated in the training of some of the staff on study procedures. YS trained and supervised clinicians and staff on study procedures and clinically evaluated and investigated patients and maintained quality assurance over clinical procedures. PKO supervised the laboratory work. AW was involved in centre coordination, data collection, and quality checks. OTI and OO were involved in clinical evaluation and investigation of the study participants, trained study team staff on procedures and maintained quality assurance over clinical procedures. BK was involved in project oversight, laboratory work supervision and interpretation of results. BMG contributed to the study design, development of the study protocol, analysis plan, interpretation of the results, and writing the final report. MAl was involved in planning, designing, and reviewing the reported study and interpretation of the results. MT was involved in planning, designing, and reviewing the reported study and statistical analysis of the data. KS was involved in the generation of study data and in the interpretation of the results. VV contributed to study design, review of the analysis plan, interpretation of the results, and laboratory work supervision. KD was involved in interpretation of the results, coordination, and reporting of the study. DB was involved in planning, designing, and reviewing the reported study, analysis plan, interpretation of the results, safety (interaction with and reporting to the Data and Safety Monitoring Board/Independent Data Monitoring Committee), project oversight, and writing the final report.

All authors had full access to all data in the study, contributed to the writing of this report, and had final responsibility for the decision to submit for publication.

Conflict of interests

MAn, EOO, YS, PKO, AW, OTI, OO, and MAl have no conflicts of interest to disclose. AO received support for study-related travel to conferences from the GSK group of companies. BK's institution received grant from Pfizer and PATH outside the submitted work. BMG reports a grant from PATH to the LSHTM to support the study. MT is employed by the GSK group of companies. MOCO, KS, VV, and DB are employed by the GSK group of companies and own shares of the GSK group of companies. KD was employed by and owns shares of the GSK group of companies. VV is the inventor of some pending patents owned by the GSK group of companies in the pneumococcal vaccine field. The GSK group of companies, MRC, and LSHTM employees report a grant from PATH to their institutions for the conduct of this study.

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All authors attest they meet the ICMJE criteria for authorship.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.033.

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