RESEARCH PAPER

Immunogenicity and Safety of a DTaP-IPV//PRP~T Vaccine (Pentaxim) Booster Dose During the Second Year of Life in Indian Children Primed with the Same Vaccine

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Objective: To evaluate the immunogenicity and safety of a pentavalent (diphtheria, tetanus, acellular pertussis, inactivated poliovirus, Hib polysaccharide-conjugate) combination vaccine booster dose.

Design: Multicenter, open, Phase III clinical study.

Setting: Two tertiary-care hospitals in Delhi and Vellore, India.

Participants/patients: 207 healthy Indian children.

Intervention: The DTaP-IPV//PR~NT vaccine (Pentaxim) was given at 18-19 months of age to children who had been primed with the same vaccine at 6,10,14 weeks of age.

Main outcome measures: Immunogenicity was assessed before and 1 month after the booster. Safety was evaluated from parental reports, and investigator assessments.

Results: At 18-19 months of age, before boosting, the SP rates against diphtheria, tetanus, poliovirus and PRP were 82.3-100%; 90.0% of participants had anti-PRP ≥0.15 µg/mL. Anti-poliovirus titers were ≥1:8 dilution in 97.9-98.4% of participants. Anti-PT and FHA titers (≥5 EU/mL) were detectable in 82.5% and 90.8% of participants, respectively. One month after the booster dose, SP rates were 99.5% for PRP (≥1.0 µg/mL), 100% for diphtheria, tetanus (≥0.1 IU/mL) and polioviruses (≥8:1/dilution). Sero-conversion (4 fold post-booster increase in anti-PT and -FHA concentration) occurred in 96.8% and 91.7%, respectively. Geometric mean concentrations (GMC) increased from 11.7 to 353.1 EU/mL and from 18.2 to 363.4 EU/mL for anti-PT and anti-FHA, respectively. Anti-PRP GMC increased from 1.75 to 70.5 µg/mL. Vaccine reactogenicity was low; severe solicited reactions were reported by <1.4% of participants.

Conclusion: The DTaP-IPV//PRP-T vaccine booster at 18-19 months of age was well tolerated and induced strong antibody responses.

Key words: *Antibody persistence, Booster vaccination, Efficacy, Immunogenicity, Pentavalent vaccine, Safety.*

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The Indian Academy of Pediatrics (IAP) recommends *Haemophilus influenzae* type b (Hib) vaccination and IPV for all children [1]. Booster doses of many childhood vaccines, including pertussis, Hib and polio are included in many national programmes during the second year of life [2]. The primary reasons for this are persistence of pertussis and Hib disease in children in countries without routine booster vaccinations, and observation that vaccine-induced immunity wanes over time, especially when an infant primary series is not followed-up with a toddler booster vaccination [2-4]. The WHO recommends a pertussis booster for children aged 1-6 years, preferably during the second year of life, with the primary series plus booster expected to ensure protection for 6 years [5].

The safety and immunogenicity of DTaP-IPV/1PRP~T vaccine (Pentaxim) have been assessed previously [6,7]. This study evaluated the immunogenicity, and safety of a DTaP-IPV//PRP~T booster vaccination administered at 18-19 months of age in a group of children who had been given a three dose primary series vaccination of the same vaccine at 6, 10, and 14 weeks of age and monovalent hepatitis B (HB) vaccine at birth, 6 and 14 or 6, 10 and 14 weeks of age [8].

Accompanying Editorial: Pages 787.

Methods

This Phase III, open clinical study was performed at Lady Hardinge Medical College and Associated Hospitals in New Delhi and Christian Medical College Hospital, Vellore, Tamil Nadu. The study protocol and consent form were approved by each institutional review board. The study conformed to local regulations, Good Clinical Practices (GCP) and applicable International Conference on Harmonization (ICH) guidelines and the ethical principles of the Declaration of Helsinki. Written

informed consent was obtained from a parent/legal guardian of each participant before enrolment.

Healthy full-term (≥37 weeks) infants weighing ≥2.5 kg at birth who had completed primary vaccination with the DTaP-IPV//PRP~T vaccine at 6, 10, and 14 weeks of age [8] were eligible for booster vaccination with the same vaccine at 18-19 months of age. The booster phase was conducted from July 2007 to April 2008. The objectives were to measure antibody persistence prior to the booster dose and the immune response 1 month post-booster.

The composition of each 0.5 mL dose of the DTaP-IPV//PRP~T study vaccine (Pentaxim, Sanofi Pasteur, France, batch number A2053) is described elsewhere [6, 8]. The lyophilized PRP~T antigen was reconstituted with the liquid DTaP-IPV vaccine immediately before IM injection into the anterolateral aspect of the upper right thigh. Blood samples (4 mL) were collected for antibody determination just before, and 4-6 weeks after the booster. Serologic analyses were performed at Sanofi Pasteur's Global Clinical Immunology central laboratory in Swiftwater, Pennsylvania, USA, using analysis methods described elsewhere [8]. The predefined antibody levels for seroprotection (SP) were: anti-PRP ≥0.15 and ≥1.0 µg/mL, anti-poliovirus ≥8 (1/dilution), anti-diphtheria ≥0.01 and ≥0.10 IU/mL, anti-tetanus ≥0.01 and ≥0.10 IU/mL. Seroconversion (SC) for anti-pertussis antigens was defined as a ≥4-fold increase in antibody concentration post-vaccination [9].

Investigators monitored each participant for immediate adverse events for 30 minutes after vaccination. Parents/legal guardians recorded, and graded the severity of, solicited injection site (redness, swelling and tenderness) and systemic (fever - axillary temperature ≥37.4ºC, vomiting, abnormal crying, drowsiness, loss of appetite and irritability) reactions on diary cards for 8 days after vaccination. Unsolicited reactions were recorded, with onset date, intensity and resolution, for 30 days after vaccination. Serious adverse events (SAEs) were reported throughout the study.

Statistical analysis: SP and SC rates were calculated with 95% confidence intervals (CIs) using the exact binomial method. Geometric mean titers (GMTs) and concentrations (GMCs) were calculated with 95% CIs using the normal approximation. Reverse Cumulative Distribution Curves (RCDCs) for pre- and post-vaccination antibody titers were derived for each antibody response.

RESULTS

Of the 216 participants who completed the primary series, three withdrew voluntarily before the booster was given, one was lost to follow up, and five had protocol violations (received a non-study DTP vaccine). The remaining 207 participants received the booster injection and provided the first blood sample. One additional participant withdrew voluntarily before collection of the second blood sample and was excluded from the post-booster immunogenicity analysis set presented here. All 207 participants given the booster vaccination were included in the safety analysis set.

Immunogenicity: Seroprotection rates were high at 18-19 months of age when the booster dose was given (***Table* I**).

TABLE I SEROPROTECTION AND SEROCONVERSION RATES FOR EACH ANTIGEN AT 1 MONTH POST-PRIMARY, PRE-BOOSTER AND 1 MONTH POST-BOOSTER VACCINATION

Criteria	*Post-primary % (95% CI)*	*Pre-booster % (95% CI)*	*Post-booster % (95% CI)*
Anti-PRP ≥0.15 µg/mL	98.5 (95.7; 99.7)	90.0 (85.0; 93.8)	100.0 (98.2; 100.0)
Anti-PRP ≥1.0 µg/mL	89.6 (84.5; 93.4)	60.0 (52.9; 66.8)	99.5 (97.3; 100.0)
Anti-Diphtheria ≥0.01 IU/mL	99.0 (96.5; 99.9)	82.3 (76.3; 87.4)	100.0 (98.2; 100.0)
Anti-Diphtheria ≥0.10 IU/mL	18.3 (13,2; 24.4)	14.1 (9.6; 19.8)	98.0 (95.0; 995)
Anti-Tetanus ≥0.01 IU/mL	100.0 (98.2; 100.0)	100.0 (98.0; 100.0)	100.0 (98.2; 100.0)
Anti-Tetanus ≥0. 10 IU/mL	100 (98.2; 100.0)	84.2 (78.2; 89.2)	100 (98.2; 100.0)
Anti-Polio 1 ≥8 1/dil.	100.0 (98.2; 100.0)	98.4 (95.5; 99.7)	100.0 (98.2; 100.0)
Anti-Polio 2 ≥8 1/dil.	99.0 (96.5; 99.9)	97.9 (94.6; 99.4)	100.0 (98.1; 100.0)
Anti-Polio 3 ≥8 1/dil.	100.0 (98.2; 100.0)	98.4 (95.5; 99.7)	100.0 (98.1; 100.0)
Anti-PT ≥4-fold increase	94.4 (90.2; 97.2)*		96.8 (93.2; 98.8)†
Anti-FHA ≥4-fold increase	86.0 (80.4; 90.5)*		91.7 (86.8; 95.2)†

**Increase from pre-to post-priming;* †*Increase from pre-booster.*

TABLE II GEOMETRIC MEAN CONCENTRATIONS (GMCS) AND TITERS (GMTS) FOR EACH ANTIGEN AT 1 MONTH POST-PRIMARY, PRE-BOOSTER AND 1-MONTH POST-BOOSTER VACCINATION

	Post-primary GMC or GMT† (95% CI)*	*Pre-booster GMC or GMT (95% CI)*	*Post-booster GMC or GMT (95% CI)*	*Post-/pre-booster GMR(95% CI)*
Anti-PRP µg/mL	4.19 (3.52;4.98)	1.75 (1.34;2.29)	70.56 (60.22;82.67)	39.7 (29.85;52.7)
Anti-Diphtheria IU/mL	0.046 (0.040;0.053)	0.028 (0.023;0.034)	3.940 (3.286;4.723)	141.3 (117.2;170.4)
Anti-Tetanus IU/mL	0.93 (0.86;1.00)	0.29 (0.24;0.34)	13.91(12.51;15.46)	48.0 (39.8;57.8)
Anti-Polio 1 (1/dil)	435.7 (359.4;528.3)	334.4 (249.6;448.1)	7777.0 (6705.8;9019.3)	25.4 (18.8;34.2)
Anti-Polio 2 (1/dil)	447.9 (349.9;573.2)	357.4 (263.0;485.7)	8638.3 (7352.4;10149.1)	26.8 (19.0;37.7)
Anti-Polio 3 (1/dil)	1488.3 (1255.6;1764.0)	271.9 (207.1;357.0)	11523.6 (9785.4;13570.7)	50.4 (37.8;67.2)
Anti-PT EU/mL	324.2 (296.0;355.1)	11.7 (10.1;13.6)	353.1 (320.9;388.6)	29.7 (25.4;34.7)
Anti-FHA EU/mL	92.8 (83.8;102.8)	18.2 (15.1;21.9)	363.4 (324.2;407.3)	20.3 (17.0;24.4)

**Geometric mean concentration: Anti-PRP, Anti-Tetanus, Anti-Diphtheria, Anti-PT, Anti-FHA; †Geomentric mean titer, Anti-Polio; GMR=geometric mean ratio.*

At least 97.9% of the participants still had anti-tetanus concentrations ≥0.01 IU/mL and poliovirus titers ≥8 (1/dilution). Anti-diphtheria concentrations ≥0.01 IU/mL and anti-PRP concentrations ≥0.15 µg/mL were still observed in 82.3% and 90.0% of participants, respectively. Following booster vaccination, SP rates against diphtheria and tetanus (≥0.1 IU/mL) and poliovirus (≥8 1/dilution) were 98.0 to 100%; anti-PRP titers ≥1.0 µg/mL were observed in 99.5% of participants, and at least 91.7% of participants seroconverted against PT and FHA.

GMTs decreased between the primary series and booster administration (***Table* II**); however, at least 90.0% of participants were still seroprotected against tetanus (≥0.01 IU/mL), the three polioviruses (≥8 1/dilution), and Hib (anti-PRP ≥0.15 µg/mL). Seroprotective anti-diphtheria antibody concentrations (≥0.01 IU/mL) were observed in the majority of participants, although the percentage was lower than for other antigens. Anti–PT and anti- FHA concentrations ≥5 EU/mL were observed in 82.5% and 90.8% of participants, respectively (data not shown). ***Figure* 1** shows strong, linear increases for anti-PT,-FHA, -PRP, and all three -polioviruses.

Reactogenicity and safety: 87 of the 207 participants (42.0%) reported a solicited reaction within 8 days of vaccination. Most occurred within three days and resolved without treatment. The most frequent injection site reaction was tenderness (21.7%) and the most frequent systemic reactions was fever (19.3%) (***Table* III**). Unsolicited events were reported by 27 participants (13%). Most were infections (11.1% of participants) with upper respiratory tract infections (8.2% of participants) predominating. A single SAE was reported - a case of

TABLE III SOLICITED REACTIONS IN AVAILABLE INFANTS (*N*=207) WITHIN 8 DAYS AFTER A BOOSTER DOSE GIVEN AT 18-19 MONTHS OF AGE

		Number	*% (95%CI)*
Injection site reactions			
Tenderness	Any	45	21.7 (16.3; 28.0)
	Severe	2	1.0 (0.1; 3.4)
Redness	Any	18	8.7 (5.2; 13.4)
	Severe	1	0.5 (0.0; 2.7)
Swelling	Any	23	11.1 (7.2; 16.2)
	Severe	0	0.0 (0.0; 1.8)
Systemic reactions			
Fever	Any	40	19.3 (14.2; 25.4)
	Severe	3	1.4 (0.3; 4.2)
Vomiting	Any	15	7.2 (4.1; 11.7)
	Severe	1	0.5 (0.0; 2.7)
Abnormal crying	Any	22	10.6 (6.8; 15.6)
	Severe	1	0.5 (0.0; 2.7)
Drowsiness	Any	18	8.7 (5.2; 13.4)
	Severe	0	(0.0; 2.7)
Loss of appetite	Any	20	9.7 (6.0; 14.5)
	Severe	1	0.5 (0.0; 2.7)
Irritability	Any	25	12.1 (8.0; 17.3)
	Severe	0	0.0 (0.0; 1.8)

% = percentage of participants with a specific adverse event. Mild, moderate or severe tenderness: 'minor reaction when injection site is touched', 'cries and protests when injection site is touched', and 'cries when injected limb is moved, or the movement of the limb is reduced'. Erythema and swelling: a diameter of <2.5 cm was mild, 2.5-5 cm was moderate and >5 cm was severe. Mild, moderate and severe fever: axillary temperatures ≥37.4ºC to 37.9ºC, ≥38ºC to 38.9ºC, and ≥39ºC, respectively.

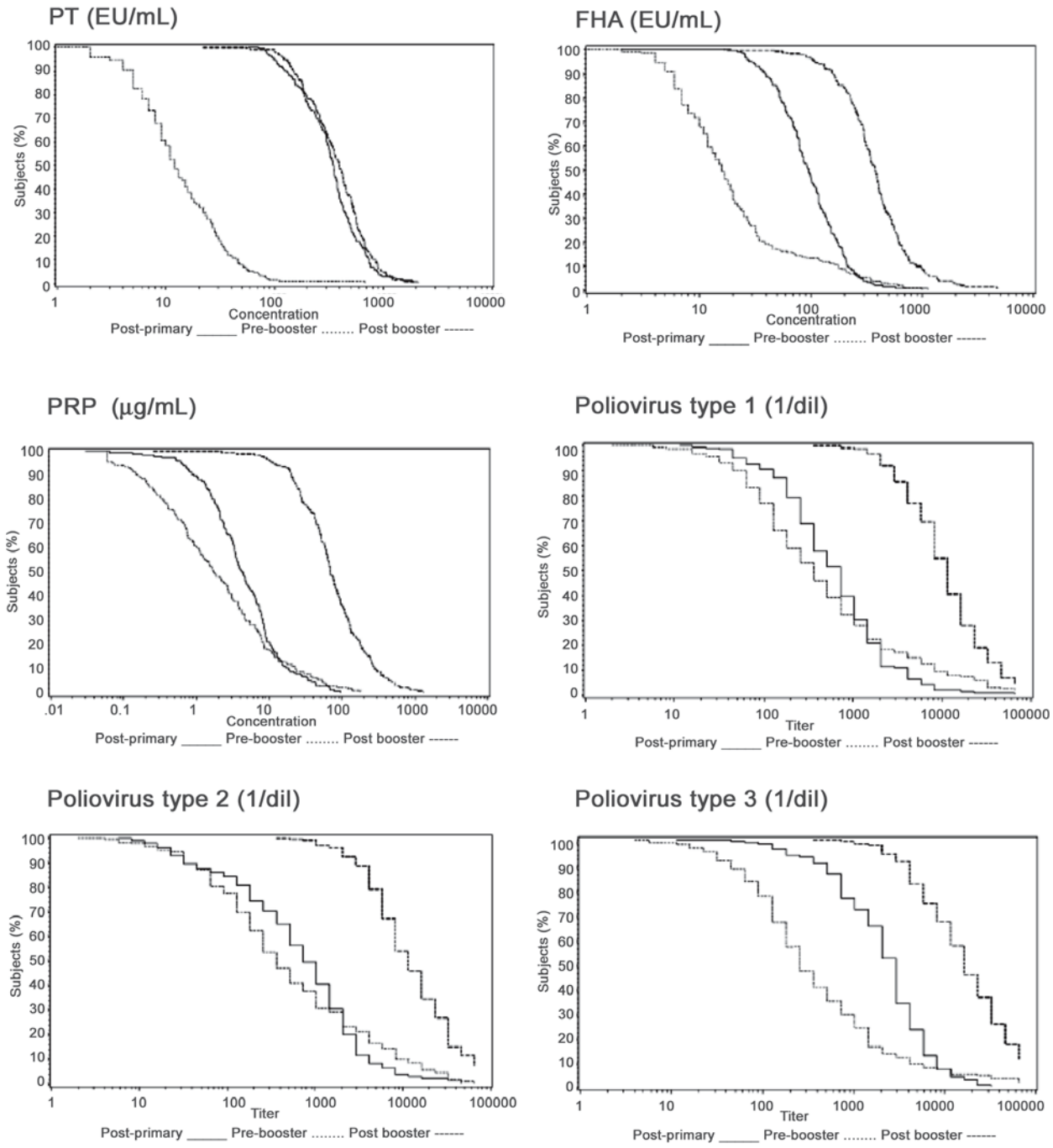

FIG.1 *Reverse cumulative distribution curves for PT, FHA, PRP, and poliovirus 1, 2, and 3 after a 3-dose primary series, and before and after a booster vaccination.*

lobar pneumonia that resolved after treatment.

DISCUSSION

This study evaluated the immunogenicity and safety of a DTaP-IPV//PRP~T vaccine booster at 18-19 months of age in participants who had completed a primary series vaccination at 6, 10, 14 weeks of age with the same vaccine given with a monovalent HB vaccine. The results following booster vaccination in this study population are consistent with previous studies of this pentavalent vaccine using various schedules, including the EPI schedule followed here [6].

WHAT IS ALREADY KNOWN?

- DTaP-IPV//PRP~T vaccine shows good antibody persistence in the second year of life and is safe and immunogenic when administered as a booster during the second year of life.

WHAT THIS STUDY ADDS?

- Additional persistence and booster immunogenicity and safety data for a DTaP-IPV//PRP~T vaccine following vaccination at 18-19 months of age in Indian children, who had received a primary series with the same vaccine at 6,10, and 14 weeks.

The very high SP rates observed here for each vaccine antigen after the booster dose, and the large increases in GMCs/GMTs, are consistent with long-term protection. The waning of anti-PT and anti-FHA serum antibody concentrations followed by a strong booster response as seen here is well documented [10,11]. Similar results have been previously reported with this and other DTaP-combined vaccines [6,12]. In this study, the post-booster SC rates of 96.8% and 91.7% for anti-PT and anti-FHA as well as the large increases in other antibody GMCs and GMTs are indicative of strong anamnestic immune responses. The anti-poliovirus antibody persistence and strong IPV booster response observed here provide additional immunogenicity data to support IPV administration in a 6, 10, 14 week EPI schedule with a booster at 18-19 months of age.

High vaccine effectiveness of DTaP combination vaccines containing conjugated Hib antigens has been demonstrated in Europe [13,14,15]. In Sweden, where the study vaccine has been in the National Program since 1997, the incidence of invasive Hib disease was 0.5/100,000 in 1997 and 0.16/100,000 in 2008 [13]. Pertussis surveillance in Sweden revealed that vaccination at 3, 5 and 12 months of age since 1997 resulted in a marked decrease in pertussis incidence compared to no vaccination. Protection has remained high for 5-7 years after the third (booster) dose, when an additional booster dose is now recommended [16,17,18]. Although the schedule followed in India is different, we believe that the Swedish surveillance data are applicable because of the high immunogenicity of this vaccine across a range of primary series and booster vaccination schedules [6,7].

Acellular pertussis vaccines are generally better tolerated than DTwP combinations for both primary and booster vaccination, but the occurrence and severity of injection site reactions tend to increase with each successive dose of either vaccine [2,5,6]. Although the incidence of solicited adverse reactions in this study was slightly higher than seen with primary vaccination, the overall reactogenicity of the booster dose indicates it was well tolerated. Severe injection site reactions occurred in no more than 7.2% of participants; no severe solicited systemic event was reported by more than 3.3%. No hypotonic-hyporesponsive episode or seizure was reported, and no participant withdrew because of a vaccination-related AE.

This study confirms that the booster at 18-19 months of age with the study vaccine was appropriately timed (with pre-booster antibody titers being satisfactory), well tolerated, and induced strong antibody responses to all the vaccine antigens.

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Competing interests: EO is employee of Sanofi Pasteur, which manufactures the vaccine evaluated in this paper.

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