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A toddler PCV booster dose following 3 infancy priming doses increases circulating serotype-specific IGG levels but does not increase protection against carriage

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ABSTRACT

Background: We compared PCV7 serological response and protection against carriage in infants receiving 3 doses (2, 4, 6 months; 3+0 schedule) to those receiving a booster (12 months; 3+1).

Methods: A prospective, randomized controlled study, conducted between 2005 and 2008, before PCVs were implemented in Israel. Healthy infants were randomized 1:1:1 to receive 3+1, 3+0 and 0+2 (control group; 12, 18 months doses). Nasopharyngeal/oropharyngeal swabs were obtained at all visits. Serum serotype-specific IgG concentrations and opsonic activities (OPA) were measured at 2, 7, 13 and 19 months. This study was registered with Current Controlled Trials, Ltd. ISRCTN28445844.

Results: Overall, 544 infants were enrolled: 3+1 (n = 178), 3+0 (n = 178) and 0+2 (n = 188).

Post-priming (7 months), antibody concentrations were similar in both groups, except for serotype 18C (higher in 3+0). Post-booster (13, 19 months), ELISA and OPA levels were significantly higher in 3+1 than in 3+0 group.

Nasopharyngeal/oropharyngeal cultures were positive for *Streptococcus pneumoniae* in 2673 (54.3%) visits. Acquisition rates (vaccine and non-vaccine serotypes) were similar for 3+1 and 3+0 groups at 7–30 months and for 0+2 group at 19–30 months.

Conclusions: PCV7 booster after 3 priming doses increased substantially IgG concentrations but did not further reduced vaccine-serotype nasopharyngeal acquisition, suggesting that protection from pneumococcal carriage does not depend primarily on serum IgG.

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1. Introduction

The 7-valent CRM₁₉₇ pneumococcal conjugate vaccine (PCV7) was licensed in the US in the early 2000s for use as 3 infant doses and a booster in second year of life (3+1 schedule) [1]. Subsequently, many countries introduced pneumococcal conjugate vaccines (PCVs) into their national immunization program (NIP) [2,3].

Abbreviations: 7VT+6A, pneumococcal serotypes included in PCV7 and serotype 6A; MCHCs, Mother-and-Child Health Centers; MOPA, multiplexed opsonophagocytosis assay; NIP, national immunization program; OPA, opsonic activities; PCVs, pneumococcal conjugate vaccines; PCV7, 7-valent CRM₁₉₇ pneumococcal conjugate vaccine; PCV13, 13-valent conjugate vaccine.

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However, considerations regarding the high cost of PCVs and the growing number of injections in infant vaccination regimens led many countries to choose different (reduced) schedules with no toddler dose (3+0) and 2 infant doses with a toddler booster dose (2+1) [4].

Schedules with a lower number of doses often elicit lower immune response compared to the original 3+1 schedule [5–11]. Furthermore, a higher nasopharyngeal carriage rate with decreasing number of PCV doses in infants was observed in several studies [6,12,13]. However, the clinical consequences of the immunologic differences are not entirely clear and are subject to debate [10,11]. Systematic reviews support a 3+0 schedule, which in various studies was typically used in infants at 6, 10, and 14 weeks or 2, 4, and 6 months schedules (the latter was often studied compared with a booster toddler dose as a 3+1 schedule) [10,14].

Reduction in nasopharyngeal carriage of pneumococcal serotypes included in PCV7 and serotype 6A (7VT+6A) plays an important role in the success of immunization programs, by reducing transmission to susceptible subjects, resulting in indirect (herd) protection [15,16]. In the US, a near total elimination of 7VT+6A was observed in all ages in the PCV7 era [17]. Several studies have shown that a 3+0 regimen was also associated with carriage reduction [8,13,18–20]. Furthermore, after the introduction of the 3+0 PCV7 regimen to the NIP in Australia, indirect protection was demonstrated [21]. However, these reports do not enable comparison of the 3+0 and 3+1 regimens, and to determine whether the 3+0 regimen is equivalent to the 3+1 regimen in its ability to induce and maintain reduction in 7VT+6A pneumococcal serotypes in the community. Furthermore, a recent study from Australia suggested that after 13-valent conjugated vaccine (PCV13) introduction with the 3+0 regimen, a lower herd protection than expected was observed, prompting a change to a 3+1 regimen [22].

We conducted a prospective randomized controlled study prior to PCV7 implementation in Israel to examine the added value of the booster dose in protecting against carriage in toddlers who had received 3 infancy doses by comparing the effect of the 3+0 and 3+1 responses on acquisition and prevalence of pneumococcal carriage in infants and toddlers. We also included in this group of children those who were not vaccinated in the first year of life, but received a 2-dose regimen at 12 and 18 months (0+2), to assess the toddler-only schedule on carriage, as this schedule only protects directly against infections caused by vaccine-serotypes in toddlers and does not protect directly against infections in infants.

2. Methods

2.1. Setting

Two ethnic groups whose socioeconomic conditions and lifestyles differ inhabit Southern Israel (the Negev region): the Jewish and the Bedouin populations. The Bedouin population, formerly desert nomads, in transition to a western lifestyle, is characterized by overcrowding, lower levels of education, lower income and larger family size than the Jewish population [23]. Pneumococcal nasopharyngeal carriage is usually higher among Bedouin infants than among Jewish infants [24]. Approximately 95% of all routine immunizations in Israel are given in public sector Mother-and-Child Health Centers (MCHCs). In the present study, we selected 7 MCHCs, 4 serving the Jewish population and 3 serving the Bedouin population (yearly birth cohorts of ~1400 and ~1700, respectively).

PCV7 was licensed in Israel in 2006 and became publicly available in 2007. However, in southern Israel, only <10% and <25% of children received the vaccine before 2008 and between 2008 and mid-2009, respectively. In July 2009, PCV7 was implemented in the Israeli NIP.

2.2. Participants

Healthy infants were enrolled at the age of 2 months (± 3 weeks). Exclusion criteria were: prematurity (<35 weeks); fever ($>38^\circ\text{C}$); congenital abnormalities; metabolic disorders; coagulation disorders; use of immune-modifying drugs; allergy to ≥ 1 vaccine constituents; hypotonic-hyporesponsiveness or persistent inconsolable crying after any prior vaccine; and HIV infection.

The study was approved by the Ethics Committees of the Soroka University Medical Center, Maccabi Health Services and the Ministry of Health and was registered with Current Controlled Trials, Ltd. ISRCTN28445844.

2.3. Study design

This open-label study was initiated in August 2005. Enrollment was conducted through March 2008, and the last follow-up visit was in March 2009, before the implementation of PCV7 in the Israeli NIP. We invited ~3100 eligible infants who belonged to the study MCHCs to take part in the study. The parents of 1141 infants were willing to consider participation in the study, and eventually 733 infants were enrolled [12]. After an informed consent was obtained, subjects were randomized 1:1:1:1 to receive 3 PCV7 doses at 2, 4, 6 months and a 4th toddler dose at 12 months (3+1; $n = 178$); 3 PCV7 doses at 2, 4, 6 months with no additional toddler doses (3+0; $n = 178$); and no dose during infancy but 2 doses in the second year at 12, 18 months (control group, 0+2; $n = 188$) (Fig. 1) [12]. The third group, originally studied as a “catch-up” schedule for the purpose of PCV7 national immunization plan introduction [12] was chosen as a control group for both the 3+1 and 3+0 groups, since they did not receive any PCV7 dose during their first year of life. An additional 189 subjects were enrolled to a 2+1 arm (presented in a previous article) [12].

The study vaccine was Prevenar™ (Wyeth Vaccines; Collegeville, PA, USA) containing 7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) individually conjugated to CRM₁₉₇; lots used were no. A94431D, B08636C, 808672K and C659732). All infants received concomitant vaccines.

Blood (2–4 mL) was drawn at ages 2, 7, 13 and 19 months, transferred refrigerated within 8 h to the laboratory, separated within 24 h and kept at -70°C until processed. In group 0+2, subjects were randomized (1:1) to give blood samples at 2 months or at 7 months of age, but all gave blood at 13 and 19 months.

Nasopharyngeal and oropharyngeal swabs were obtained at each visit using transport swabs (nasopharyngeal – MW173 Amies transport medium, Transwab, Medical Wire and Equipment, Pottery, UK; oropharyngeal – Culture-Swab Transport System, Venturi Transystem®, Copan Innovation, Italy). The swabs were kept at room temperature and processed within 16 h. Results of the oropharyngeal swabs for positive pneumococcal culture were used only if the nasopharyngeal swabs were culture-negative. Pneumococcal identification and serotyping were performed as previously described [25].

2.4. Serologic assays

Serum serotype-specific pneumococcal anti-capsular IgG concentrations for PCV7 serotypes (4, 6A, 6B, 9V, 14, 18C, 19F and 23F) were measured using ELISA at the Department of Applied Immunology & Endocrinology, Kinder und Jugendklinik Universitätsklinikum Erlangen, Germany, after double absorption with C-polysaccharide and pneumococcal serotype 22F polysaccharide [5,26–28].

The opsonic activities of anti-pneumococcal antibodies were measured against PCV7 serotypes by a fourfold multiplexed opsonophagocytosis assay (MOPA) as previously described [29], with minor modifications [30].

Serum samples from which enough serum volume was available ($n = 281$) were submitted also to MOPA assay (in addition to ELISA assay). For those samples, ELISA GMCs were compared to MOPA GMTs. Since serotype 6A antigen is not a part of PCV7, serotype 6A MOPA was compared to serotype 6B ELISA antibodies.

2.5. Data and statistical analysis

Data were analyzed with SPSS 18.0 software for Windows (Chicago, IL, USA). Contingency table analyses were performed using χ^2 test or Fisher's exact test. Continuous variables were analyzed using the *t*-test or ANOVA procedures. Correction for ethnicity

Age (m)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	30
PCV7 3+1																										
PCV7 3+0																										
PCV7 0+2																										
Blood sample			✓					✓						✓						✓						
NP/OP culture			✓		✓		✓	✓					✓	✓					✓	✓					✓	✓
Concomitant Vaccines	HBV	HBV	DTaP-IPV-PRPT		DTaP-IPV-PRPT		DTaP-IPV-PRPT	HBV					DTaP-IPV-PRPT + MMR						HAV						HAV	

Fig. 1. Study design. NP, nasopharyngeal; OP, oropharyngeal. At ages 2, 4, 6 and 12 months – diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b and inactivated poliovirus vaccine (DTaP-IPV-PRPT, GlaxoSmithKline Biologicals, Rixensart, Belgium); at ages 12 months – measles, mumps and rubella vaccine (MMR; GlaxoSmithKline Biologicals Rixensart, Belgium). Hepatitis B vaccine (HBV) was given outside the study visits at ages 0, 1, and 7 months. Hepatitis A vaccine (HAV) was given outside the study visits at ages 18, and 24 months.

was done using Mantel-Haenszel or multi-regression analysis. A P -value $< .05$ was considered significant.

For the post-primary series, the proportion of subjects with serotype-specific IgG concentrations ≥ 0.35 and > 1.0 $\mu\text{g/mL}$ were calculated. For ages 13 and 19 months the proportion of subjects with concentration > 1.0 and > 5.0 $\mu\text{g/mL}$ were calculated. The latter was associated in previous publications with protection against carriage [31,32]. Additional comparisons between groups were conducted using $\log_{10}$ -transformed geometric mean concentrations (GMCs).

Since protection against serotype 6A may be obtained through antibodies elicited by serotype 6B antigen [33], we combined serotype 6A in the analysis with 7VT.

In a previous study [5], we demonstrated that although Bedouin infants had higher maternally-derived serotype-specific serum IgG than the Jewish infants for all serotypes, after the infant immunization series, no significant difference between the 2 ethnic groups was observed. Nevertheless, all serology results were adjusted for ethnic group. Furthermore, in another study, ethnicity did not

Fig. 2. Flow diagram for the study groups. NP, nasopharyngeal; OP, oropharyngeal. The 0+2 group was randomized 1:1 to have a blood sample done either at 2 or 7 months. SD = Standard Deviation.

Table 1
Serum serotype-specific pneumococcal anticapsular IgG GMCs ($\mu\text{g/mL}$) and percent of subjects achieving serum concentrations ≥ 0.35 , >1.0 and $>5.0 \mu\text{g/mL}$ at 7, 13 and 19 months of age.

Serotype		7 months				13 months				19 months			
		3+1 n = 145	3+0 n = 157	0+2 n = 86	P	3+1 n = 145	3+0 n = 152	0+2 n = 156	P	3+1 n = 146	3+0 n = 139	0+2 n = 141	P [*]
4	GMC (95% CI)	1.9 (1.6;2.3)	2.0 (1.7;2.2)	0.0 (0.0;0.0)	β_2, γ_2	4.0 (3.4;4.7)	0.3 (0.3;0.4)	2.5 (2.1;3.1)	$\alpha_2, \beta_2, \gamma_2$	0.4 (0.4;0.5)	0.1 (0.1;0.2)	4.4 (3.7;5.3)	$\alpha_2, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	98.6	98.1	2.3	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	77.9	79.6	1.2	β_2, γ_2	91.0	9.9	78.1	$\alpha_2, \beta_1, \gamma_2$	81.4	46.8	100	$\alpha_2, \beta_2, \gamma_1$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	42.8	0.0	30.1	$\alpha_2, \beta_1, \gamma_2$	19.9	10.8	89.4	$\alpha_1, \beta_2, \gamma_2$
6B	GMC (95% CI)	2.3 (1.8;3.0)	1.9 (1.4;2.4)	0.1 (0.1;0.1)	β_2, γ_2	11.0 (8.8;13.8)	0.8 (0.6;1.0)	0.4 (0.3;0.5)	$\alpha_2, \beta_2, \gamma_2$	2.0 (1.6;2.4)	0.8 (0.6;0.9)	2.9 (2.3;3.7)	$\alpha_2, \beta_1, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	89.7	83.4	8.1	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	72.9	70.7	1.2	β_2, γ_2	93.1	40.8	25.2	$\alpha_2, \beta_2, \gamma_1$	23.3	5.0	87.2	$\alpha_2, \beta_2, \gamma_2$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	79.3	9.2	2.6	$\alpha_2, \beta_2, \gamma_1$	0.7	0.7	15.7	β_2, γ_2
9 V	GMC (95% CI)	1.5 (1.2;1.7)	1.4 (1.3;1.6)	0.1 (0.1;0.1)	β_2, γ_2	3.5 (3.0;4.0)	0.5 (0.4;0.5)	2.0 (1.7;2.4)	$\alpha_2, \beta_2, \gamma_2$	0.7 (0.6;0.8)	0.3 (0.3;0.4)	3.6 (3.0;4.2)	$\alpha_2, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	96.6	96.2	4.7	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	64.1	66.0	2.3	β_2, γ_2	92.4	16.4	78.7	$\alpha_2, \beta_1, \gamma_2$	43.8	30.2	85.8	$\alpha_1, \beta_2, \gamma_2$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	33.1	0.0	18.1	$\alpha_2, \beta_1, \gamma_2$	14.4	10.8	55.3	β_2, γ_2
14	GMC (95% CI)	5.2 (4.2;6.5)	5.0 (4.2;6.1)	0.1 (0.0;0.1)	β_2, γ_2	12.9 (11.0;15.2)	1.4 (1.1;1.7)	2.1 (1.7;2.6)	$\alpha_2, \beta_2, \gamma_1$	2.4 (2.0;2.8)	0.9 (0.7;1.1)	15.4 (13.2;17.9)	$\alpha_2, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	93.8	95.5	20.9	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	88.3	87.3	4.7	β_2, γ_2	98.6	61.2	73.1	$\alpha_2, \beta_2, \gamma_1$	46.9	8.6	80.7	$\alpha_2, \beta_2, \gamma_2$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	84.1	11.2	23.7	$\alpha_2, \beta_2, \gamma_1$	6.2	0.7	27.7	$\alpha_1, \beta_2, \gamma_2$
18C	GMC (95% CI)	1.5 (1.3;1.8)	1.7 (1.5;1.9)	0.0 (0.0;0.0)	β_2, γ_2	3.7 (3.2;4.3)	0.3 (0.3;0.4)	1.9 (1.6;2.3)	$\alpha_2, \beta_2, \gamma_2$	0.5 (0.4;0.6)	0.2 (0.2;0.2)	2.3 (2.0;2.7)	$\alpha_2, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	93.8	98.7	1.2	$\alpha_1, \beta_2, \gamma_2$	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	72.7	73.5	1.2	β_2, γ_2	91.0	8.6	75.0	$\alpha_2, \beta_2, \gamma_2$	15.8	1.4	91.4	$\alpha_2, \beta_2, \gamma_2$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	38.6	0.7	18.6	$\alpha_2, \beta_2, \gamma_2$	2.7	0.7	52.5	β_2, γ_2
19F	GMC (95% CI)	1.8 (1.5;2.2)	2.1 (1.8;2.5)	0.0 (0.0;0.0)	β_2, γ_2	4.1 (3.4;4.9)	0.5 (0.4;0.7)	0.4 (0.4;0.5)	α_2, β_2	1.0 (0.8;1.3)	0.6 (0.5;0.8)	5.0 (3.9;6.3)	$\alpha_1, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	91	94.9	1.2	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	74.5	78.3	1.2	β_2, γ_2	89.0	23.0	26.9	α_2, β_2	73.1	37.0	75.2	α_2, γ_2
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	46.2	8.6	3.2	$\alpha_2, \beta_2, \gamma_1$	19.9	7.2	39.7	$\alpha_1, \beta_2, \gamma_2$
23F	GMC (95% CI)	1.3 (1.0;1.5)	1.0 (0.9;1.3)	0.1 (0.0;0.1)	β_2, γ_2	5.6 (4.7;6.7)	0.4 (0.3;0.5)	0.6 (0.5;0.7)	$\alpha_2, \beta_2, \gamma_1$	0.9 (0.7;1.1)	0.3 (0.2;0.3)	2.8 (2.2;3.4)	$\alpha_2, \beta_2, \gamma_2$
	% $\geq 0.35 \mu\text{g/mL}$	88.3	82.2	4.7	β_2, γ_2	ND	ND	ND	ND	ND	ND	ND	ND
	% $>1.0 \mu\text{g/mL}$	57.6	51.9	2.3	β_2, γ_2	95.9	19.7	32.3	$\alpha_2, \beta_2, \gamma_1$	29.5	8.6	91.4	$\alpha_2, \beta_2, \gamma_2$
	% $>5.0 \mu\text{g/mL}$	ND	ND	ND	ND	54.9	1.3	5.1	α_2, β_2	2.7	1.4	34.0	β_2, γ_2

α , 3+1 vs 3+0 group: $\alpha_1, .001 \leq P < .05$; $\alpha_2, P < .001$.

β , 3+1 vs 0+2 group: $\beta_1, .001 \leq P < .05$; $\beta_2, P < .001$.

γ , 3+0 vs 0+2 group: $\gamma_1, .001 \leq P < .05$; $\gamma_2, P < .001$.

ND = Not done.

* P value adjusted for ethnic group.

influence the association between IgG concentrations and prevention of carriage despite differences in carriage rates [34].

New pneumococcal acquisition was defined as acquisition of a serotype not seen at any previous visit. New acquisitions were calculated from 7 months of age (one month following complete infant series) onwards. Cumulative incidences were calculated by adding the incidence of a certain point (adjusted for ethnicity) to all previous rates within the same group. All acquisitions and prevalence rates are expressed per 100 children.

3. Results

Overall, ~3100 eligible infants were invited to take part in the study. The parents of 1141 were willing to consider participation in the study, and 544 agreed to be enrolled: 3+1 ($n = 178$), 3+0 ($n = 178$) and 0+2 ($n = 188$) (Fig. 2). At all visits, the mean age was similar in all groups. Bedouin infants constituted 50% and females constituted 48% of subjects, with no significant differences in these distributions between the 3+1, 3+0 and 0+2 groups.

3.1. Immunogenicity

At 7 months of age, no significant differences in immunogenicity between the 3+0 and 3+1 groups were observed, except for serotype 18C, with a higher proportion of infants with antibody concentrations $\geq 0.35 \mu\text{g/ml}$ in the 3+0 group (Table 1). For all serotypes, the antibody concentrations were higher among both 3+1 and 3+0 groups compared to the 0+2 group.

At 13 months, the antibody concentrations were significantly higher in the 3+1 than in the 3+0 group for all serotypes. The 0+2 group (after 1 dose at 12 months) had higher antibody concentrations than the 3+0 group for all serotypes, except 19F and 6B, and lower antibody concentrations than the 3+1 group for all serotypes. At age 19 months, one month after the second PCV7 dose for group 0+2, the antibody concentrations in the 0+2 group were higher for all serotypes compared to both 3+1 and 3+0 groups. The concentrations in the 3+1 group were all significantly higher than those in the 3+0 group.

Trends for ELISA GMCs paralleled those of MOPA GMTs for each serotype and study age group (Fig. 3).

3.2. Nasopharyngeal/oropharyngeal new acquisition rates

Overall, nasopharyngeal/oropharyngeal (NP/OP) cultures samples were obtained in 4925 visits: 1616, 1628 and 1681 for the 3+1, 3+0, and 0+2 groups, respectively (Fig. 2). Cultures were positive for *S. pneumoniae* in 2673 (54.3%) visits; of these, 0.6% were positive from the OP sample only. A total of 496 (10.1%) new acquisitions of 7VT+6A were observed: 6A (123, 2.5%), 19F (112, 2.4%), 6B (78, 1.6%), 23F (77, 1.6%), 14 (55, 1.1%), 18C (25, 0.5%), 9V (20, 0.4%) and 4 (6, 0.1%). Serotypes 6A and 6B constituted together 40.5% of all new acquisitions.

3.2.1. Comparison between the 3+1, the 3+0 and the 0+2 groups

Acquisition of 7VT+6A (combined) was similar for groups 3+1 and 3+0 at all ages from 7 to 30 months, with no significant differences in any of the age points (Fig. 4A). P-values in Fig. 4 reflect the

Fig. 3. Comparison between ELISA IgG geometric mean concentrations (GMCs, $\mu\text{g/ml}$) and MOPA geometric mean titers (GMTs) at ages 7, 13 and 19 m between groups 3+1, 3+0 and 0+2. The values are given with 95% confidence intervals. The tested serotypes were 4, 6A, 6B, 9V, 14, 18C, 19F and 23F. A. ELISA IgG GMCs ($\mu\text{g/ml}$). B. MOPA GMTs. *Since serotype 6A antigen is not a part of PCV7, serotype 6A MOPA GMTs were compared to serotype 6B ELISA GMCs. Statistical analyses are presented in Table 1.

Fig. 4. Comparison of cumulative incidence (per 100 children) of new pneumococcal NP/OP acquisitions between the 3+1 and 3+0 groups. These 2 groups were in turn compared to the 0+2 groups, who received no PCV7 during the first year of life, but received 2 doses in the second year of life. (A) PCV7 serotypes (including serotype 6A). (B) Serotype 6B separately. (C) Serotype 6A separately. (D) Non-PCV7 serotypes (excluding serotype 6A). All P-values were adjusted for ethnic groups (Jewish vs. Bedouin children). α P-values between the 3+1 and 3+0 groups. β P-values between the 3+1 and 0+2 groups. γ P-value between the 3+0 and 0+2 groups. The denominators used for the calculation of the incidence rates presented in Fig. 4 are detailed in Fig. 2.

rate ratio (adjusted for ethnicity) between age-specific cumulative incidences.). Both groups differed significantly from the 0+2 group at ages 7–12 months (a period during which no dose was administered to the 0+2 group), and ages 13–18 months (after 1 dose at 12 months among the 0+2 group). However, at ages 19–30 months (after group 0+2 received 2 PCV doses), no significant differences between the 0+2 groups and the 3+1 or 3+0 group were observed. Several differences were observed for serotypes 6B separately and 6A separately (Fig. 4B and C). These include higher cumulative incidences of serotype 6B in the 0+2 group compared with the 3+0 group at ages 7–12 months and the 3+1 and 3+0 groups at ages 13–18 months. Similarly, for serotype 6A, higher cumulative incidences in the 0+2 group were observed compared with the 3+1 and 3+0 groups at ages 13–18 months. For non 7VT+6A serotypes, no difference was observed between groups 3+1 and 3+0, but for the 3+0 group acquisitions of non VT7 at ages 7–18 months was significantly higher than the group 0+2 (Fig. 4D).

3.3. Prevalence rates of nasopharyngeal/oropharyngeal carriage

At any time point, and for all comparisons, no significant differences between groups 3+1 and 3+0 in the mean carriage

prevalence were observed (Fig. 5). However, for all 7VT+6A combined, and serotypes 6B and 6A separately, the mean carriage prevalence in groups 3+0 and 3+1 was generally lower than that in the 0+2 group. This difference was statistically significant at ages 7+12 and 13+8 months for 7VT+6A combined; all ages for serotype 6B; and age 13+18 months for serotype 6A.

For non-VT+6A, the mean carriage prevalence was significantly higher in the 3+1 and 3+0 groups than in the 0+2 group at ages 7+12 and 13+18 months. No significant differences were observed for overall pneumococcal carriage prevalence between all groups.

4. Discussion

In the current study, when PCV7 was administered to infants at a 3-dose schedule with a 2-month interval (2, 4 and 6 months of age), adding a further dose at 12 months of age markedly and significantly boosted serotype-specific anticapsular IgG responses to all 7 serotypes and OPA titers to all measured serotypes, resulting in higher concentrations than without a booster for at least 6 months. However, paradoxically, no significant added benefit was observed in term of both nasopharyngeal acquisition and preva-

Fig. 5. Mean prevalence during visits conducted at 7 and 12 months (grouped), 13 and 18 months (grouped and 19, 24 and 30 months (grouped)), by serotypes and serotype groups. Mean prevalence was calculated as the mean of prevalence in each of the visits in the grouped intervals. (A) PCV7 serotypes (including serotype 6A). (B) Serotype 6B separately. (C) Serotype 6A separately. (D) non-PCV7 serotypes (excluding serotype 6A). (E) Total pneumococcal carriage. α P-values between the 3+1 and 3+0 groups. β P-values between the 3+1 and 0+2 groups. γ P-value between the 3+0 and 0+2 groups.

lence of VT7+6A serotypes throughout all visits up to 30 months of age. To the best of our knowledge, this is the only study that compared the efficacy of 3+0 vs. 3+1 regimens on carriage with a relatively long follow-up (age 2.5 years).

These findings are unique for several reasons. First, while 3+0 PCV schedules were studied in several countries worldwide and both efficacy/effectiveness against both invasive pneumococcal disease [22] and nasopharyngeal carriage [35], and impact on these outcomes were shown, none of these studies were compared head-to-head to the 3+1 regimen. To our knowledge, this is the first study that compared 3+1 and 3+0 schedules within a comparative randomized trial. Furthermore, the study was conducted in Israel during a period when PCVs were not part of the NIP. This is of crucial importance, since without such a head-to-head study, it is difficult to assess the real difference in efficacy of the different schedules against nasopharyngeal carriage. Second, our findings are reassuring and suggest that reduced schedules may impact equally both direct and indirect protection through reduced VT7 spread. Reduced schedules are indeed desirable for several reasons, including the high cost of PCVs and to limit the growing number of injections in infant vaccination. Third, while a higher nasopharyngeal carriage rate with decreasing number of PCV doses in infants was observed in several studies [8,12,13], our results demonstrate that the number of doses may play a smaller role than previously thought in reducing carriage, at least in a schedule with a 2-

month interval between doses. Whether similar results can be obtained with a shorter interval between doses (i.e. the 6, 10, 14 week interval used in developed countries), is not clear.

The lack of direct correlation between the post-booster serum antibody and carriage acquisition and prevalence is somewhat surprising. The following can be a plausible explanation. Correlation between serotype-specific IgG concentrations and nasopharyngeal acquisition of pneumococcus post infant 3-dose primary series was clearly demonstrated [33,34]. It may be speculated that high blood IgG concentration is not, by itself, sufficient for the prevention of carriage acquisition. Protection against carriage may require an additional stimulation of the immune system, building a higher number of memory B-cells. This hypothesis lends support from a recent human challenge study demonstrating that indeed, serotype-specific blood memory B-cells correlated with nasopharyngeal carriage, while circulating serotype-specific IgG or IgA levels or even functional capacity of serum antibody were not associated with protection against carriage [36]. In this case, we can speculate that PCV7 sufficiently stimulated specific memory B cells after 3 doses to protect against nasopharyngeal carriage acquisition of vaccine-serotype pneumococcus. Adding booster at 12 months of age, although resulting in boosting circulating antibody concentration, was not needed in the presence of the critical number of memory B cells. In our study, the protection against carriage without the booster was similar to that of the booster up to age 30

months, and we did not examine further durations of protection. It is thus not clear whether the booster dose further stimulated the memory B-cell potentially resulting in longer protection than without a booster. This necessitates a longer follow-up, with additional studies needed measuring both B cell memory and antibodies concentration in correlation to protection against carriage for longer durations.

Other than the differences in the immunogenicity of the 3+1 and 3+0 schedules, one additional major concern regarding the use of reduced schedules is the possibility of lower indirect (herd) protection, as was observed in Australia compared to the United Kingdom and the United States, following PCV13 implementation [22]. The study raised questions about the serotypes included PCV13 beyond those in PCV7 and whether the longevity of protection against carriage of the additional PCV13 serotypes could not be shorter than that of the PCV7 serotypes. Since the key for successful herd protection is the reduction of new carriage acquisition, the question raised by the Australian experience emphasized that choosing 3+0 over 3+1 schedule should be accompanied with strict monitoring of pneumococcal disease rates in both vaccinated and non-vaccinated populations (both children >5 years and adults). Indeed, a transient increase in non-PCV7 serotypes carriage rate was observed in our study in the 3+0 vs. the 0+2 schedules (but not when the 3+1 and the 0+2 schedules were compared), again emphasizing the importance of strict monitoring when choosing 3+0 over 3+1 schedule.

The data from our controls (the 0+2 schedule) are important. In the 0+2 group, antibodies concentration and carriage acquisition rates were favorable with the 3+1 and the 3+0 groups, when evaluated in the 2nd and the 3rd years of life. However, in the 0+2 schedule, infants (<12 months) are not directly protected against vaccine-type acquisition of carriage. This point is important in view of recent thought to potentially implement regimens with only 1 dose in infancy and a second later in life (booster at 9 months of age), relying on herd protection derived from reduction of cases.

The main strengths of our study include the relatively large number of subjects, the methodology of randomized, controlled trial and the fact that the study was conducted in the pre-PCV licensure era. Additionally, the relatively long-term follow-up for carriage enabled appreciation of the efficacy up to 2.5 years post the last dose of the 3+0 regimen.

The main limitation of the current study is that only PCV7+6A serotypes were evaluated (rather than PCV13 or PCV10 serotypes, currently in use).

In conclusion, while a toddler booster PCV7 dose increased antibody concentrations, in the currently tested regimen of 2, 4 and 6 months, the added benefit of the booster dose to reduce carriage of PCV7+6A serotypes was not shown. This suggests that protection against nasopharyngeal acquisition of *S. pneumoniae* does not necessarily depend on circulating IgG, but may also depend on other factors, such as the presence of circulating or submucosal memory B-cells.

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