



Safety and immunogenicity of CRM197-conjugated pneumococcal–meningococcal C combination vaccine (9vPnC–MnCC) whether given in two or three primary doses^{☆,☆☆}

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ABSTRACT

This randomized trial compares safety and immunogenicity when vaccinating infants with a pneumococcal–meningococcal conjugate vaccine in two doses vs. three doses. Infants ($N = 223$) received 9vPnC–MnCC (CRM197-conjugated pneumococcal serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F and meningococcal C polysaccharides) either at 3 and 5 or 3, 4 and 5 months and a booster with either 9vPnC–MnCC or 23-valent pneumococcal-polysaccharide vaccine (23vPPS) and CRM197–MnCC, at 12 months. Safety was monitored and IgG measured at 3, 6, 12 and 13 months in all subjects and serum bactericidal activity (SBA) in half. The 9vPnC–MnCC vaccine was safe and induced significant IgG to all components. Three doses induced higher antibody GMCs (geometric mean concentrations) at 6 months to seven of nine pneumococcal serotypes. This was most significant for 6B and 23F ($p < 0.001$), that also showed lower rate of responders >0.35 (6B, 23F) and $>0.5 \mu\text{g}/\text{mL}$ (6B). Antibody GMCs remained lower following 9vPnC–MnCC booster in subjects primed with two doses although only significant for serotype 18C. Significant memory responses were observed 1 week after the 23vPPS toddler dose. MnCC–IgG GMC was lower after two doses, however with comparable SBA. This study shows that the 9vPnC–MnCC vaccine is safe and induces successful immunological memory, whether given in two or three primary doses.

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1. Introduction

Protein conjugated polysaccharide vaccines against *Haemophilus influenzae* type B [1], *Streptococcus pneumoniae* [2–6] and *Meningococcus* type C [7] have proven immunogenic in infants and protective against invasive disease. Since licensure of Prevnar[®], a 7-valent pneumococcal CRM197 conjugate, in the USA,

a dramatic decrease has been observed in invasive pneumococcal disease caused by the vaccine serotypes, in vaccinated as well as non-vaccinated children and adults, demonstrating a herd immunity [8,9]. The extremely high efficacy (97.5%) against invasive pneumococcal disease (IPD) in the Kaiser Permanente efficacy trial raised a hope that problems of antibiotic resistance would be reduced and that childhood mortality in the developing countries could be decreased by infant vaccination [2,3,10]. In the efficacy trial in the Gambia the 9-valent pneumococcal CRM197 conjugate (tested in the current study of 9vPnC–MnCC vaccine) showed 37% efficacy against first episode of radiological pneumonia, 77% against IPD caused by vaccine serotypes and 16% against mortality [4]. In the double-blinded infant trial in Finland, otitis media caused by the seven vaccine type pneumococci was reduced by 57% and by all pneumococci by 34% [11]. Prevnar[®] is licensed to be administered in three primary doses with a toddler dose to boost the immunity. In the Nordic countries routine infant immu-

[☆] The study was presented at the 23rd annual ESPID in Valencia, Spain May 18–20, 2005 [Abstract # P15B; <http://www.kenes.com/epid2005/program/abstracts/288.doc>] and at the 5th ISPPD, Alice Spring, Australia, April 2–6, 2006 [Abstract# SY10.05].

^{☆☆} Clinical trial registration URL: <http://www.personuvernd.is>, registration # 454, and the National Bioethics Committee, <http://www.visindasidanefnd.is> #01097AG2.

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nizations are given at 3 and 5 months of age with a booster at 12 months. Results from an open, nonrandomized, multi-centre study in Sweden may indicate that Prevenar® could be administered in two primary doses, which would make its introduction for routine vaccination more acceptable for families and more economical. Until now, the kinetics of antibody responses within 1 month after booster vaccination with pneumococcal conjugate vaccine or pneumococcal-polysaccharides has not been reported. When measured 4 weeks after booster, higher GMC–IgG responses were observed to 23-valent polysaccharide vaccine than diphtheria or tetanus conjugated pneumococcal-polysaccharides [12], but on the other hand lower avidity has been reported [13,14].

In this randomized clinical trial we investigated the safety and immunogenicity of a 9-valent CRM197-conjugated pneumococcal-polysaccharide vaccine combined with a CRM197-conjugated Meningococcus C polysaccharide. We compared two primary doses vs. three primary doses in infancy, followed by a booster vaccination with either the same conjugate or 23-valent pneumococcal-polysaccharide vaccine co-administered with MnC-CRM127 at 12 months. We investigated further the kinetics of the booster response in a subgroup of children by measuring the specific IgG antibodies before, 1 and 4 weeks after the toddler vaccination, comparing groups that received two primary doses vs. three primary doses.

2. Methods

2.1. Study design

This randomized study evaluated safety and immunogenicity of a combined 9-valent pneumococcal and meningococcal C conjugate vaccine, administered according to either a two- or a three-dose primary immunization schedule, followed by a booster dose. The study was approved by the National Bioethics Committee (#01097AG2) and the Data Protection Authority (#454) in October 2001. An informed consent was obtained from the parents and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Healthy, term infants were recruited during well childcare at three health centres in Iceland: Center for Child Health in Reykjavik, Solvangur in Hafnarfjordur and Borgir in Kopavogur.

The primary objective of the study was to evaluate the difference in pneumococcal antibody concentrations following primary immunization at either 3 and 5 months of age or at 3, 4 and 5 months of age. Booster responses following either the 9-valent pneumococcal, meningococcal C conjugate combination vaccine or the 23-valent polysaccharide pneumococcal vaccine at 12 months, were evaluated in both primary immunization groups as a secondary objective. Kinetics of antibody responses to the nine pneumococcal serotypes and Meningococcus group C were finally explored 1 week after the booster dose in all groups.

2.1.1. Vaccines

The trial vaccine contained nine pneumococcal serotype polysaccharides, 2 µg of saccharide per pneumococcal serotypes 1, 4, 5, 9V, 14, 18C, 19F and 23F, 4 µg of pneumococcal serotype 6B and 10 µg of meningococcal group C oligosaccharide (same concentration as in monovalent Meningococcus C CRM197 conjugate, Meningitec®) coupled to 18.5 µg of CRM197 carrier protein. Each 0.5 mL dose contained a total of approximately 38.5 µg of CRM197 carrier protein and 0.5 mg of aluminium phosphate (0.125 mg elemental aluminium) as adjuvant. For booster vaccination, either the same conjugate vaccine or 23-valent pneumococcal-polysaccharide vaccine (23VPPS) (Pneumo23®, Sanofi Pasteur

MSD; *S. pneumoniae* serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F; 25 µg each) and CRM197-conjugated meningococcal C polysaccharide (MnCC) (Meningitec®, Wyeth AB; *Neisseria meningitidis*; 10 µg oligosaccharide serotype C11 coupled to 15 µg CRM197 carrier protein).

The concomitant vaccines, DTaP-IPV/Hib (Pentavac®, Sanofi Pasteur MSD) were administered in the right thigh according to the Icelandic recommendations, at 3, 5 and 12 months of age.

2.1.2. Vaccination schedules

The 9vPnC–MnCC vaccine was administered in the left thigh either at 3 and 5 months of age ($n = 112$; two-dose group) or 3, 4 and 5 months ($n = 111$; three-dose group). To compare the priming by these two schedules the children were randomized to receive a booster of either 9vPnC–MnCC or 23VPPS and MnCC at 12 months.

2.1.3. Safety

Local reactions, systemic events and body temperature were recorded by parents on diary cards for 7 days after each vaccine dose. Adverse events were monitored for 28 days after the primary series doses and the booster dose.

2.1.4. Blood samples

Blood samples were obtained at 3, 6, 12 and 13 months of age. To explore the kinetics of the booster response, a blood sample was obtained from a subgroup of children ($N = 61$) whose parents were willing to bring them in for an extra visit 1 week after booster immunization at 12 months. Specific IgG was measured in all samples at all time points and serum meningococcal bactericidal activity was measured in sera from half of the subjects 4 weeks after the primary series (6 months) and the booster dose (13 months).

2.2. Measurements

Serum was separated within 8 h and kept at -80°C until IgG antibodies to pneumococcal serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F and meningococcal C polysaccharides were measured by ELISA as described elsewhere [15–18]. In the pneumococcal ELISA protocol Cell Wall Polysaccharide neutralization was performed but serotype 22F pre-adsorption was not. The antibody levels were calculated from the reference standard 89SF and expressed in µg/mL. Meningococcal serum bactericidal activity (SBA) assay was performed on a randomly selected subset of subjects (60 per group) [15,19]. All measurements were done by Wyeth Vaccines Research at ARUP in Salt Lake City, Utah, USA.

2.3. Expression of results and statistics

The study was powered to answer the primary objective by recruiting 224 infants. Subjects included in the immunogenicity analysis were those immunized per protocol with blood samples drawn per protocol at 3, 6, 12 and 13 months of age.

The exploratory results on post-booster antibody kinetics were obtained from 61 infants who came for an extra blood sample 1 week after booster. The data presented as exploratory were evaluated separately and obtained from the same subjects at 12 months, 12 months plus 1 week and 13 months. We used all available subjects out of the 61, and by excluding those who did not comply with the time limits the results did not change.

The results for each primary series schedule group are expressed as geometric mean concentration (GMC) and 95% confidence interval (C.I.) of IgG antibodies (µg/mL) and geometric mean and 95% C.I. SBA titre. The ratio of the GMCs between the two primary series schedule groups and its 90% confidence interval was determined based on the log-transformed antibody levels of the two groups.

Table 1
Number (%) of children completing each infant vaccination schedule and toddler dose

	Two-dose infant schedule	Three-dose infant schedule
Completed infant series	111 (99%)	111 (100%)
Available for immunogenicity	108 (96%)	110 (99%)
Received toddler dose	110 (98%)	108 (98%)
9vPnC–MnCC toddler dose	53 (48%)	52 (48%)
Available for immunogenicity	45 (41%)	46 (43%)
23v PPS toddler dose + MnCC	57 (52%)	56 (52%)
Available for immunogenicity	47 (43%)	48 (44%)

The difference of the means in log-scale and the 90% confidence interval derived from the *t*-statistic was transformed back to obtain the GMC ratio and corresponding confidence interval.

Percentage exceeding 0.35 and 0.5 µg/mL of IgG pneumococcal antibody concentration, and MnC SBA titre reaching 1:128 at 6 and 13 months was calculated for each group and reverse cumulative distribution curves constructed. Ninety-five percent confidence interval was determined for the difference of percent responders between the two primary series schedule groups.

Frequencies of adverse events were compared between the two schedule groups using Fisher's exact test.

3. Results

3.1. Recruitment, compliance and analysis groups

Out of 223 infants recruited, 112 were randomized to receive two infant doses (two-dose group) and 111 three doses (three-dose group) of the 9vPnC–MnCC vaccine at 3 and 5 or 3, 4 and 5 months of age, respectively. Of these 111 (99.1%) in the two-dose group and 111 (100%) in the three-dose group completed the infant primary vaccination series, one infant in the two-dose group was lost to follow-up. Total of 108 in the two-dose group and 110 in the three-dose group were evaluated for immunogenicity at 6 months leaving out 4 and 1 infants, respectively, who did not comply with the per protocol time limits. Distribution of the infants and toddlers completing each phase is shown in Table 1. For the 32 toddlers excluded for toddler immunogenicity analysis, serum was not available or they did not comply with the protocol time limits. Gender and age distributions were comparable between the treatment groups at all timepoints.

Table 2
Serotype specific IgG responses after two or three primary vaccinations with 9vPnC–MnCC at 3 and 5 months (Two-dose infant schedule) or 3, 4 and 5 months (Three-dose infant schedule)

Serotype	GMC (95% C.I.)		<i>p</i> ^a -Values: two doses vs. three doses	Ratio of GMCs two-dose schedule/three-dose schedule (90% C.I. ^a)	Percent subjects with >0.35 µg/mL (95% C.I.)		<i>p</i> ^b -Values: two doses vs. three doses
	Two-dose infant schedule N = 108	Three-dose infant schedule N = 110			Two-dose infant schedule N = 108	Three-dose infant schedule N = 110	
1	3.62 (3.13, 4.19)	3.34 (2.93, 3.80)	0.494	1.08 (0.92, 1.28)	100.0 (96.6, 100.0)	100.0 (96.7, 100.0)	1
4	2.34 (2.01, 2.74)	2.97 (2.62, 3.38)	0.016	0.79 (0.67, 0.93)	100.0 (96.6, 100.0)	100.0 (96.7, 100.0)	1
5	1.20 (1.02, 1.41)	1.52 (1.33, 1.74)	0.020	0.79 (0.66, 0.94)	94.4 (88.3, 97.9)	97.3 (92.2, 99.4)	0.3324
6B	0.69 (0.52, 0.90)	1.94 (1.48, 2.53)	<0.001	0.35 (0.26, 0.49)	62.0 (52.2, 71.2)	86.4 (78.5, 92.2)	<0.0001
9V	1.73 (1.47, 2.02)	1.99 (1.74, 2.27)	0.175	0.87 (0.73, 1.03)	99.1 (94.9, 100.0)	99.1 (95.0, 100.0)	1
14	4.69 (3.66, 6.02)	6.95 (5.82, 8.29)	0.007	0.68 (0.52, 0.87)	98.1 (93.5, 99.8)	98.2 (93.6, 99.8)	0.6832
18C	1.52 (1.33, 1.75)	1.83 (1.60, 2.09)	0.034	0.83 (0.71, 0.98)	98.1 (93.5, 99.8)	97.3 (92.2, 99.4)	1
19F	3.20 (2.65, 3.87)	4.19 (3.62, 4.84)	0.022	0.76 (0.63, 0.93)	96.3 (90.8, 99.0)	99.1 (95.0, 100.0)	0.2124
23F	0.91 (0.72, 1.14)	1.77 (1.36, 2.31)	<0.001	0.51 (0.38, 0.69)	81.5 (72.9, 88.3)	90 (82.8, 94.9)	0.058

Left panel shows post infant series IgG GMCs in µg/mL (95% C.I.) and GMC ratios (90% C.I.) between two- and three-doses groups at 6 months of age. The right panel shows the percentage exceeding 0.35 µg/mL for each vaccination schedule. *p*-Values reflect differences between the two vaccination schedules in each panel.

^a GMC and confidence interval based on two-sample *t*-test with pooled variance on log-transformed data.

^b Rate of responders compared with Fisher's exact test.

3.1.1. Exploratory evaluation

Parents of 61 infants agreed to bring their child for an extra evaluation and blood test 1 week after booster dose at 12 months. Of those, 29 received two primary 9vPnC–MnCC doses out of whom 14 and 15 received 9vPnC–MnCC or 23VPPS + MnCC toddler dose, respectively. Thirty-two received 3 primary 9vPnC–MnCC doses with 18 and 14 receiving 9vPnC–MnCC or 23VPPS + MnCC toddler dose, respectively.

3.2. Safety

Three infants experienced serious adverse events, none considered to be related to the trial vaccine. One in the two-dose group had septicemia 7 days after the second dose, caused by *S. pneumoniae* serotype 7F. The same infant presented later with fever, otitis media and asthma and was further found to be neutropenic. One other infant in the two-dose group was admitted to hospital due to asthma and the third one in the three-dose group was admitted due to viral gastroenteritis and dehydration.

3.3. Immunogenicity

3.3.1. Infant series

At 6 months, following the infant immunization series, a significant rise was observed in IgG antibodies to all the pneumococcal serotypes contained in the vaccine whether vaccinated with two or three doses of the 9vPnC–MnCC combination vaccine (*p* < 0.001). Three doses induced higher GMC IgG levels for seven out of nine serotypes, most significant for the poorly immunogenic serotypes 6B and 23F (Table 2) with GMC at 6 months for two doses vs. three doses; of 0.69 µg/mL vs. 1.94 µg/mL and 0.91 µg/mL vs. 1.77 µg/mL (*p* < 0.001 for both), respectively. The difference was significant by *t*-test for serotypes 4, 5, 14, 18C and 19F as also reflected by the ratio of GMCs for two-dose/three-dose schedules (Table 2). However, the rate of responder achieving the estimated protective antibody levels of ≥0.35 µg/mL, was 62–100% vs. 86–100% in the two-dose groups vs. three-dose groups, respectively, significantly lower for serotype 6B in the two-dose group at 62% compared to 86.4% in the three-dose group (*p* < 0.0001). Although not significant, lower percentage reached this level for serotype 23F in the two-dose group compared to the three-dose group, at 81.5% vs. 90%, respectively (*p* = 0.058) (Table 2, Fig. 1).

MnCC IgG GMC at 6 months was significantly lower after two, compared to three doses, 5.48 µg/mL vs. 10.98 µg/mL (*p* < 0.0001),

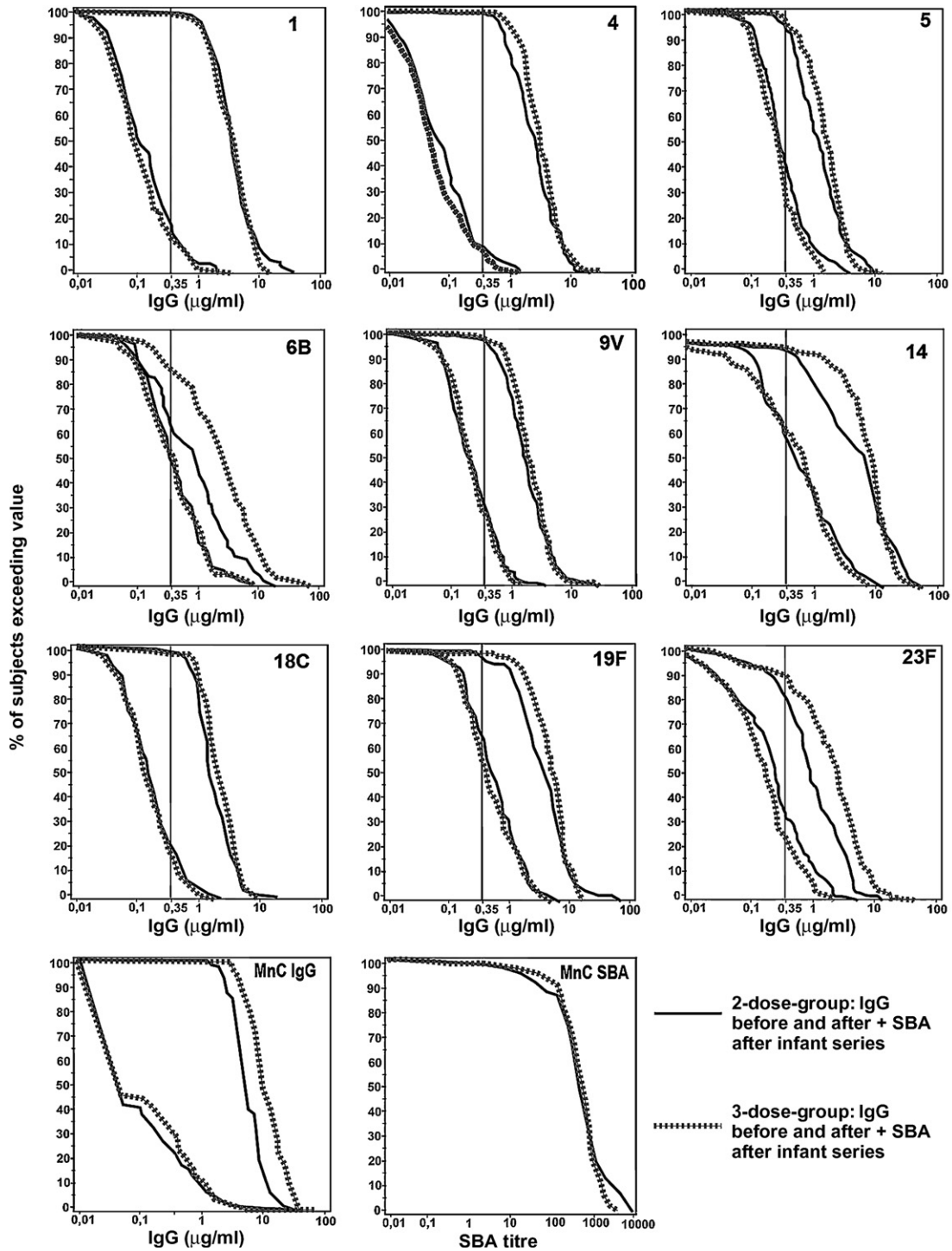


Fig. 1. Reverse cumulative distribution curves for pneumococcal serotypes, meningococcal C specific IgG and meningococcal C SBA before and after primary vaccinations at 3 and 5 or 3, 4 and 5 months. The level of >0.35 µg/mL is indicated by a vertical line.

respectively, however there was no difference in SBA titre; 572 vs. 564, respectively. Importantly, 100% of both groups reached the proposed protective SBA titer of 1:8 [20] and 87.5% vs. 93% reached the level of 1:128, in the two-dose groups vs. three-dose groups, respectively (Table 3, Fig. 1).

3.3.2. Toddler series

At 12 months of age, before the booster dose was administered, antibody levels to all serotypes had declined and there was no

difference between the two-dose and three-dose groups. Significant booster responses were observed in both groups, whether they received a booster with 9vPnC–MnCC or 23vPPS + MnCC ($p < 0.001$). A trend for lower antibody GMCs was observed following the 9vPnC–MnCC booster dose for serotypes, 6B, 14, 18C, 19F and 23F in subjects that received only two primary vaccine doses, although due to the limited sample size, only the difference for 18C reached statistical significance ($p = 0.044$). Serotype 1, on the other hand, had higher booster response in the two-dose group when

Table 3
Meningococcal C IgG responses and serum bactericidal activity at 6 months of age (1 month after primary vaccination) and at 13 months of age (1 month post-booster) according to number of infant doses and type of booster

Assay	1 month after infant series			1 month after 9vPnC–MnCC booster			1 month after 23vPPS booster			
	N	Two-dose infant schedule GMC/GMT (95% C.I.)	Three-dose infant schedule GMC/GMT (95% C.I.)	N	Two-dose infant schedule GMC/GMT (95% C.I.)	Three-dose infant schedule GMC/GMT (95% C.I.)	N	Two-dose infant schedule GMC/GMT (95% C.I.)	Three-dose infant schedule GMC/GMT (95% C.I.)	
ELISA GMC	108	5.48 (4.86, 6.18)	10.98 (9.68, 12.47) ^a	45	9.12 (7.05, 11.78)	7.74 (6.16, 9.72)	47	13.63 (10.73, 17.31) ^b	14.24 (11.04, 18.35) ^c	
SBA GMT	56	572 (372, 878)	564 (394, 807)	23	1928 (1176, 3160)	1611 (1008, 2572)	28	2623 (1595, 4313)	29	3634 (2218, 5954)
%SBA ≥ 128	56	87.5 (75.9, 94.8)	93.0 (83.0, 98.1)	23	100.0 (85.2, 100.0)	100.0 (86.8, 100.0)	28	100.0 (87.7, 100.0)	29	100.0 (88.1, 100.0)

Confidence interval based on two-sample *t*-test with pooled variance on log-transformed data.

^aDifference for two doses vs. three doses at 6 months; *p* < 0.0001. Difference between type of booster within the ^btwo-dose group; *p* = 0.009 and within the ^cthree-dose group; *p* < 0.0001.

vaccinated with to 23vPPS + MnCC (*p* = 0.022 (Table 4). Compared to the booster with the 9vPnC–MnCC conjugate, a booster vaccination with 23vPPS + MnCC resulted in significantly higher GMC IgG levels after 4 weeks for pneumococcal serotypes 1, 4, 5, 9V, 18C, and 19F in the two-dose group and for serotypes 1, 4, 9V and 19F in the three-dose group (Table 4). In contrast, higher response to serotype 6B was observed in those who received 9vPnC–MnCC booster (*p* = 0.019 and 0.016 for two- and three-dose groups, respectively). For serotypes 14 and 23F the booster responses were comparable between booster vaccine groups. For each of the pneumococcal serotypes contained in the 9vPnC–MnCC vaccine, 89–100% of the subjects had an IgG antibody concentration ≥ 0.5 µg/mL 1 month after the booster dose, regardless of number of primary doses in infancy and type of booster dose (Fig. 2).

Booster response to MnCC administered with the 23vPPS vaccine was also higher than when administered in 9vPnC–MnCC combination vaccine (*p* = 0.009 and < 0.0001 for two- and three-dose group respectively) (Table 3).

3.3.3. Kinetics of the booster response, exploratory analysis

Exploratory analysis shows the results for the subgroup of the 61 infants evaluated 1 week after the booster vaccination. The IgG results were analyzed separately for these 61 subjects at all immunogenicity time points, namely 12, 121/4 and 13 months. Both the 9vPnC–MnCC and the 23vPS + MnCC booster induced significant memory responses demonstrated by rapid rise in GMC IgG by 1 week (*p* < 0.0001), for all serotypes in all four groups. For all the pneumococcal serotypes, the booster with 9vPnC–MnCC tended to induce maximum GMC IgG antibodies 1 week after booster vaccination. In contrast, booster responses induced by the 23vPS + MnCC, reached maximum pneumococcal antibody levels at 13 months (Table 5).

MnCC specific IgG response to both 9vPnC–MnCC and MnCC booster reached also maximum level 1 week after the booster dose and subsequently declined in all the groups.

4. Discussion

In this study we have compared the safety and immunogenicity of CRM197-conjugated pneumococcal–meningococcal C combination vaccine (9vPnC–MnCC) when given in two primary doses vs. three primary doses to healthy infants at 3 and 5 or 3, 4 and 5 months of age.

The two vaccine schedules demonstrated a comparable safety profile with three subjects having five serious adverse effects, none of which was judged by the investigators to be related to the study vaccination. One infant had positive blood culture due to the non-vaccine serotype 7F, which at that time was the most prevalent pneumococcal serotype in Iceland [21]. Antibody levels to all vaccine type pneumococcal serotypes increased significantly following the infant immunization, whether administered in two or three primary doses. Looking at either antibody GMCs in both groups or at GMC ratios between groups, higher antibody levels were reached in the three-dose group. When comparing the rate of responders, the inferiority of two doses compared to three doses was seen only for serotype 6B at 0.35 and 0.5 µg/mL and for serotype 23F at 0.5 µg/mL. Although not significant at the time of booster immunization, the higher levels for serotype 6B were maintained in the three-dose group.

The current only licensed pneumococcal conjugate vaccine Prevnar[®] has proven to be effective against invasive pneumococcal disease [2]. It is licensed to be administered in three infant doses and one booster dose. Due to vaccine shortage it was administered in other schedules as well and according to the US Centres for

Table 4

Post-toddler dose IgG GMCs in $\mu\text{g/mL}$ (95% C.I.) to 9vPnC–MnCC pneumococcal serotypes for each infant schedule group according to type of booster at 12 months

Serotype	9vPnC–MnCC toddler dose			23vPPS toddler dose		
	GMC (95% C.I.)		Ratio of GMCs two-dose/three-dose group (90% C.I.)	GMC (95% C.I.)		Ratio of GMCs two-dose/three-dose group (90% C.I.)
	Two-dose infant schedule <i>N</i> = 45	Three-dose infant schedule <i>N</i> = 45		Two-dose infant schedule <i>N</i> = 47	Three-dose infant schedule <i>N</i> = 48	
1	4.48 (3.56, 5.65)	4.48 (3.48, 5.78)	1.00 (0.75, 1.33)	12.68 ^{a,***} (10.43, 15.43)	8.70 ^{***} (6.49, 11.67)	1.46 (1.09, 1.95)
4	3.87 (3.04, 4.92) ^a	4.30 (3.43, 5.40)	0.90 (0.68, 1.18)	9.98 ^{***} (8.23, 12.11)	9.40 ^{***} (7.50, 11.77)	1.06 (0.83, 1.36)
5	3.28 (2.65, 4.04)	3.18 (2.60, 3.90)	1.03 (0.81, 1.31)	5.02 ^{***} (4.10, 6.15)	4.15 (3.28, 5.26)	1.21 (0.94, 1.56)
6B	9.42 [†] (6.34, 14.00)	14.01 [†] (9.41, 20.86)	0.67 (0.42, 1.07)	5.26 (3.40, 8.14)	7.19 (4.87, 10.62)	0.73 (0.45, 1.19)
9V	2.39 (1.94, 2.95)	2.55 (2.06, 3.16)	0.94 (0.73, 1.20)	3.76 [†] (2.92, 4.86)	4.09 ^{***} (3.22, 5.21)	0.92 (0.69, 1.23)
14	8.75 (6.37, 12.02)	10.15 (8.20, 12.55)	0.86 (0.63, 1.18)	7.57 (5.22, 10.99)	9.61 (6.72, 13.73)	0.79 (0.52, 1.21)
18C	1.79 (1.43, 2.24)	2.37 ^b (1.92, 2.92)	0.76 (0.59, 0.98)	2.78 ^{***} (2.29, 3.37)	2.75 (2.10, 3.61)	1.01 (0.77, 1.33)
19F	3.83 (2.98, 4.93)	4.48 (3.38, 5.93)	0.86 (0.63, 1.17)	11.65 ^{***} (9.23, 14.72)	12.17 ^{***} (9.06, 16.36)	0.96 (0.70, 1.31)
23F	2.83 (1.90, 4.23)	4.42 (3.23, 6.06)	0.64 (0.42, 0.98)	2.92 (2.11, 4.03)	3.18 (2.07, 4.88)	0.92 (0.59, 1.43)

Comparison and confidence interval based on two-sample *t*-test on log-transformed data. Differences between two-dose groups vs. three-dose groups significant for ^aserotype 1 in PPS + MnCC booster group (*p* = 0.021) and serotype ^b18C in PnC–MnCC booster group (*p* = 0.044).

p-values within booster groups are provided where significant difference was between type of booster within each primary vaccine group: **p* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.

Disease Control and Prevention’s Active Bacterial Core surveillance on the invasive disease, the effectiveness of one or more doses against vaccine serotypes was 96% (95% C.I. 93–98) in healthy children and 81% (57–92) in those with coexisting disorders [22]. In that study a booster gave a significantly better protection than three-dose infant schedule alone.

Other studies have evaluated safety and immunogenicity of CRM197-conjugated pneumococcal vaccines when administered in two infant doses with a booster dose. In a nonrandomized Swedish cohort, all serotypes except 6B and 23F had satisfactory antibody

response to immunization with Prevenar[®] at 3 and 5 months, evaluated at 6 months like in our study, but booster responses to 6B and 23F were robust at 12 months, indicating comparable memory responses [23]. The comparison with three-dose primary schedule in that study can however be questioned since they used historical controls from studies using different concomitant vaccines [11] or different methods measuring antibody concentration [3]. Based on the memory responses at the time of the toddler dose and those historical controls, the authors conclude that two and three primary immunizations are comparable.

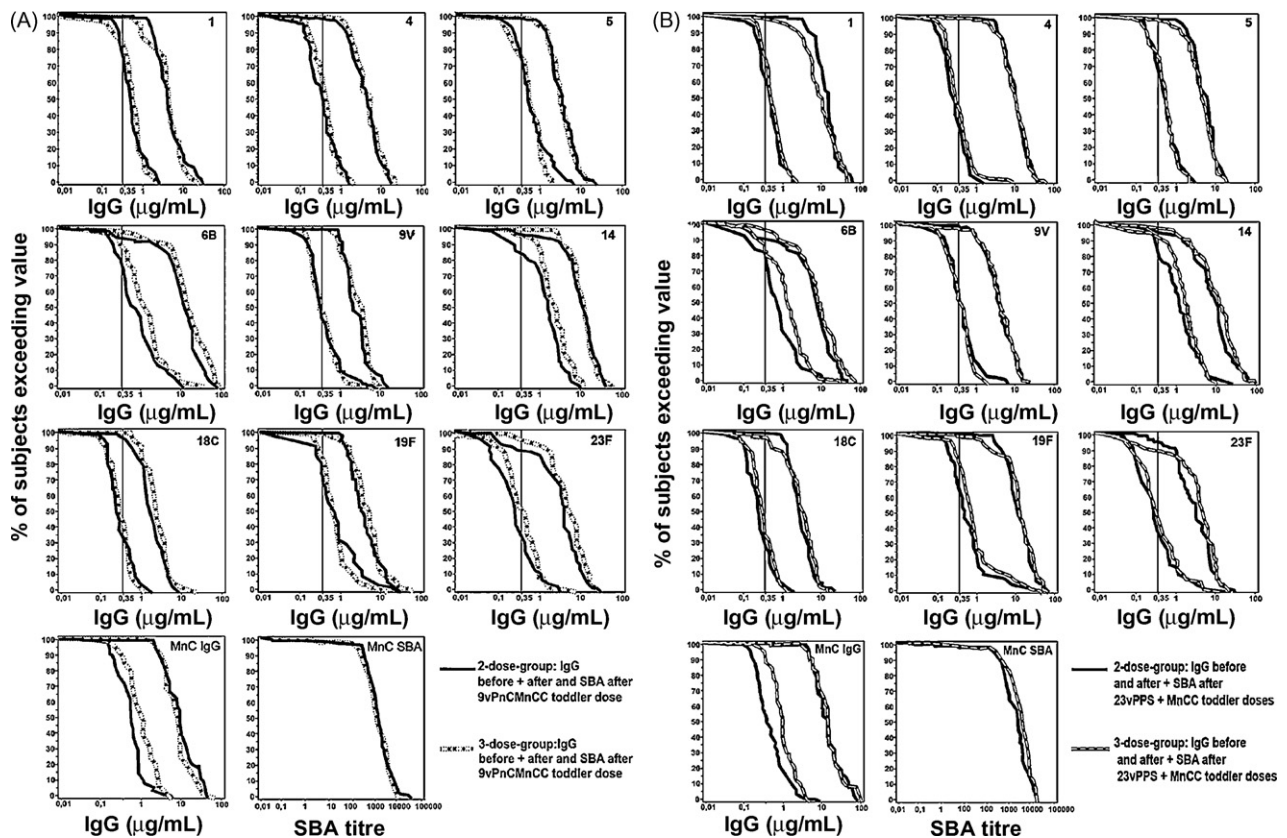


Fig. 2. Reverse cumulative distribution curves for pneumococcal serotypes, meningococcal C specific IgG and meningococcal C SBA before and after booster vaccination with 9vPnC–MnCC (panel A) and PPS + MnCC (panel B) at 12 and 13 months. The level of >0.35 $\mu\text{g/mL}$ is indicated by a vertical line.

Table 5
 Exploratory results from a subgroup of children (N = 61) who came for an extra blood drawing 1 week after booster vaccination

Serotype	9vPnC-MnCC toddler dose			23vPPS toddler dose			9vPnC-MnCC toddler dose			23vPPS toddler dose		
	Before	1 week	4 weeks	Before	1 week	4 weeks	Before	1 week	4 weeks	Before	1 week	4 weeks
1	0.71 (0.42, 1.20)	6.10 (4.28, 8.69)	5.10 (3.05, 8.54)	0.57 (0.41, 0.79)	10.54 (7.10, 15.64)	13.34 (9.05, 19.65)	0.56 (0.41, 0.77)	6.31 (4.09, 9.75)	4.88 (3.40, 7.01)	0.54 (0.36, 0.82)	5.91 (3.22, 10.86)	6.60 (3.12, 13.98)
4	0.38 (0.23, 0.62)	6.05 (3.61, 10.14)	4.65 (2.75, 7.86)	0.33 (0.24, 0.45)	5.95 (3.47, 10.21)	11.76 (7.36, 18.80)	0.39 (0.30, 0.52)	5.00 (3.64, 6.87)	3.82 (2.79, 5.25)	0.40 (0.27, 0.59)	5.15 (3.05, 8.70)	8.06 (4.60, 14.14)
5	0.87 (0.46, 1.65)	6.42 (5.11, 8.07)	4.87 (3.28, 7.34)	0.59 (0.43, 0.80)	3.42 (2.27, 5.15)	6.48 (4.50, 9.33)	0.55 (0.38, 0.80)	4.04 (2.92, 5.59)	3.34 (2.38, 4.68)	0.49 (0.29, 0.83)	2.52 (1.48, 4.27)	3.15 (1.91, 5.20)
6B	1.07 (0.54, 2.12)	15.44 (8.69, 27.42)	12.40 (7.74, 19.88)	1.07 (0.66, 1.73)	8.00 (4.82, 13.01)	9.26 (5.79, 14.82)	1.13 (0.81, 1.58)	21.23 (12.39, 36.36)	16.66 (9.81, 28.29)	1.43 (0.74, 2.74)	8.83 (4.15, 18.78)	9.84 (5.13, 18.91)
9V	0.61 (0.30, 1.22)	4.31 (3.27, 5.69)	3.23 (2.03, 5.15)	0.40 (0.24, 0.67)	3.40 (2.38, 4.86)	4.32 (2.75, 6.78)	0.41 (0.31, 0.55)	3.35 (2.35, 4.77)	2.54 (1.84, 3.51)	0.35 (0.21, 0.58)	3.14 (1.80, 5.48)	3.95 (2.13, 7.31)
14	1.64 (0.95, 2.85)	11.28 (7.61, 16.73)	9.53 (6.94, 13.08)	1.65 (0.92, 2.94)	7.55 (3.82, 14.92)	8.56 (4.42, 16.58)	1.64 (1.14, 2.35)	10.66 (7.22, 15.74)	8.72 (6.07, 12.52)	1.55 (0.74, 3.21)	5.48 (2.58, 11.68)	7.25 (3.46, 15.23)
18C	0.37 (0.23, 0.60)	3.17 (2.13, 4.71)	2.19 (1.44, 3.34)	0.28 (0.22, 0.37)	2.90 (1.95, 4.30)	2.99 (2.06, 4.34)	0.32 (0.24, 0.43)	3.25 (2.19, 4.83)	2.31 (1.65, 3.25)	0.30 (0.21, 0.43)	2.70 (1.57, 4.62)	2.39 (1.37, 4.19)
19F	1.20 (0.50, 2.91)	11.17 (7.62, 16.38)	6.59 (4.41, 9.87)	0.57 (0.39, 0.84)	3.53 (1.96, 6.34)	7.70 (3.98, 14.87)	0.80 (0.49, 1.32)	5.51 (3.22, 9.42)	3.59 (2.37, 5.44)	1.39 (0.56, 3.43)	5.44 (2.45, 12.10)	6.87 (3.20, 14.77)
23F	0.41 (0.20, 0.82)	6.23 (3.27, 11.89)	3.84 (1.82, 8.09)	0.44 (0.20, 0.97)	3.54 (2.21, 5.70)	3.20 (1.76, 5.80)	0.48 (0.30, 0.78)	8.71 (5.97, 12.70)	5.58 (3.99, 7.81)	0.46 (0.22, 0.97)	4.78 (2.93, 7.79)	4.35 (2.59, 7.29)
Men C	0.57 (0.36, 0.88)	12.43 (8.70, 17.75)	8.78 (5.98, 12.89)	0.47 (0.34, 0.66)	2.022 (13.71, 29.84)	14.13 (9.29, 21.50)	0.58 (0.66, 1.45)	10.41 (6.59, 16.45)	7.95 (5.22, 12.11)	0.85 (0.60, 1.19)	13.74 (9.03, 20.89)	11.74 (6.56, 21.03)

The table shows IgG geometric mean concentrations ($\mu\text{g/mL}$) and 95% CI, before, 1 and 4 weeks after booster vaccination with 9vPnC-MnCC or 23vPPS + MnCC. Each group is divided according to number of primary 9vPnC-MnCC doses.

In two separate cohorts, recruited consecutively at two separate sites in the UK, immunogenicity of a 9-valent pneumococcal CRM97 conjugate from the same manufacturer and at the same dose as in our study was compared when administered at 2 and 4 months or at 2, 3 and 4 months. In both studies a booster vaccination was given at 12 months with either the same conjugate or the 23-valent pneumococcal-polysaccharide vaccine. The primary responses were similar in the two-dose and three-dose groups with avidity maturation and significant booster responses measured 4 weeks after the booster dose. The percentage of infants reaching IgG concentration of 0.35 $\mu\text{g/mL}$ was also comparable for all serotypes. Similar to our study the 23vPPS booster tended to give higher GMC-IgG levels 4 weeks after the toddler dose [24].

Besides the current study comparing two primary doses vs. three primary doses of pneumococcal conjugate vaccine, there is one randomized study on 668 recruited infants, currently being conducted in Israel. In that study the investigators compare immunogenicity and nasopharyngeal carriage after two primary immunizations vs. three primary immunizations with 7-valent CRM197-conjugated pneumococcal vaccine at 2, 4 and 6 ($N=327$), at 4 and 6 months ($n=171$) or after no pneumococcal conjugate vaccination ($n=170$). In that study, the two-dose schedule induced significantly lower IgG GMC levels for serotypes 6B, 14, 18C and 23F at 7 months. The two-dose group tended to have higher acquisition of serotype 6B and 6A at 7 and 12 months compared with the three-dose group which the authors relate to the lower immunogenicity of the two-dose schedule [25].

In a prospective observational nonrandomized study in which 1571 Italian infants received Prevenar[®] and a hexavalent vaccine or hexavalent vaccine alone at 3, 5 and 11 months, the group receiving Prevenar[®] had overall significantly less otitis media and community acquired pneumonia than the group receiving hexavalent vaccine alone, indicating a protective effect of pneumococcal conjugate vaccine when administered in two primary doses with a booster, however there was no reduction in the interval period between the second and the third dose [26].

In order to decrease number of infant vaccinations by combining pneumococcal and Meningococcus C CRM197 conjugates the immunogenicity of MenC in the 9vPnC-MnCC vaccine was compared to monovalent group C meningococcal conjugate vaccine administered in addition to routine immunizations at 2, 3 and 4 months in a randomized UK study. In that study the 9vPnC-MnCC combination vaccine demonstrated reduced MnCC immunogenicity compared with the MenC conjugate alone, although without reduction in immunogenicity for the pneumococcal serotypes [27]. In our study a similar pattern was observed following the booster dose; the group receiving 23vPPS + MnCC booster vaccination demonstrated higher MnC-IgG GMC compared with the 9vPnC-MnCC booster, both 1 week ($p=0.058$) and 4 weeks ($p=0.08$) after booster, however with an excellent antibody response in both cases.

Our study is the first one to explore in toddlers the kinetics of pneumococcus specific IgG responses after a booster vaccination. The CRM197-conjugated polysaccharides induced in 1 week a very brisk memory response, exceeding what was observed in response to the native polysaccharide alone. The maximum levels measured at 1 week post conjugate booster subsequently decline significantly during the next 3 weeks opposite to the response to the polysaccharide which continued to rise until 4 weeks after the booster vaccination illustrating difference in the nature of the booster immune response to protein conjugated or native polysaccharide. Thus our results may support previously described results showing two antibody forming B-cell populations in response to protein-polysaccharide booster vaccination, due to T cell help,

while only one population may respond to the polysaccharide booster [28]. Earlier studies have demonstrated difference in quantity and avidity of booster induced specific antibodies 4 weeks after booster when induced by protein conjugated polysaccharides vs. native pneumococcal-polysaccharides [13,14], which may impact functionality. Opsonophagocytosis activity (OPA) was not measured in this study. Low avidity antibodies may result in lower OPA [29]. Further evaluation of the functionality of antibodies in the period between primary and booster vaccination, would provide valuable complementary information about the clinical relevance of the observed lower post-primary responses. However, in this study the post-booster antibody levels in all four groups are far above the protective levels for all nine serotypes and are therefore likely to provide high OPA.

Successful immunological priming is the key to protection by vaccination. It is clear that in our study, the three primary doses induced higher antibody responses in infancy and although not significant for most serotypes it tended to induce higher antibody responses to a booster dose at 1 year of age. Still, the rate of responders $>0.35 \mu\text{g/mL}$ is comparable at that age, a level that has been recommended by WHO as critical goal for vaccine success. The kinetics of the specific antibody responses clearly indicates a successful priming and induction of an immunological memory. It is also noteworthy that the serotype with the lowest immunogenicity in infancy, serotype 6B, elicits the highest response at time of booster.

Several countries have started or are planning to implement two-dose pneumococcal conjugate vaccination to their infant vaccination schedule [30–32]. These decisions may have been based on published data from nonrandomized studies showing adequate immune responses and immunological memory development but not on direct randomized comparison of two doses with the current recommended schedule of Prevenar[®]. It remains to be seen whether the inferiority of the two-dose schedule between 6 months of age and the time of booster is of clinical importance that may in fact affect the decision whether societies will advise two or three primary vaccinations.

The data from this study suggest a pneumococcal-meningococcal conjugate combination vaccine (9vPnC–MnCC) administered either as a two-dose primary infant schedule (3 and 5 months of age) or as a three dose primary infant schedule (3, 4, and 5 months of age) followed by a toddler dose at 12 months of age, is safe and induces a significant primary immune responses to both vaccination schedules, priming for similar memory responses at 12 months of age. The three-dose schedule provides higher primary antibody responses, but still very significant immune responses were observed to two-doses only that may effectively protect against invasive pneumococcal diseases with less cost. The clinical relevance of the reduced immune responses when using a two-dose primary immunization schedule, remains however unknown in terms of potential impact on protection against mucosal infections such as pneumonia and acute otitis media.

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