

Immunogenicity and Reactogenicity of a Reduced Schedule of a 4-component Capsular Group B Meningococcal Vaccine: A Randomized Controlled Trial in Infants

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Background. The 4-component capsular group B meningococcal vaccine (4CMenB) was licensed as a 4-dose infant schedule but introduced into the United Kingdom as 3 doses at 2, 4, and 12 months of age. We describe the immunogenicity and reactogenicity of the 2 + 1 schedule in infants.

Methods. Infants were randomized to receive 4CMenB with routine immunizations (test group) at 2, 4, and 12 months or 4CMenB alone at 6, 8, and 13 months of age (control group). Serum bactericidal antibody (SBA) assay against a serogroup B meningococcal reference strain (44/76-SL), memory B-cell responses to factor H binding protein, *Neisseria* adhesion protein A, *Neisseria* heparin binding antigen, Porin A (PorA), and reactogenicity was measured.

Results. One hundred eighty-seven infants were randomized (test group: 94; control group: 93). In the test group, 4CMenB induced SBA titers above the putative protective threshold (1:4) after primary and booster doses in 97% of participants. Postbooster, the SBA GMT (72.1; 95% confidence interval [CI], 51.7–100.4) was numerically higher than the serum bactericidal antibody geometric mean titre (SBA GMT) determined post–primary vaccination (48.6; 95% CI, 37.2–63.4).

After primary immunizations, memory B-cell responses did not change when compared with baseline controls, but frequencies significantly increased after booster. Higher frequency of local and systemic adverse reactions was associated with 4CMenB.

Conclusions. A reduced schedule of 4CMenB was immunogenic and established immunological memory after booster.

Keywords. 4-component capsular group B meningococcal vaccine; 4CMenB; memory B cells; immunogenicity; reduced schedule; meningococcal disease; reactogenicity.

Serogroup B *Neisseria meningitidis* (MenB) is the most common cause of meningococcal disease in Europe, accounting for 51% of the cases in 2017, with the highest rates in the infant population [1]. A 4-component capsular group B meningococcal vaccine (4CMenB) containing 4 antigens—*Neisseria* adhesion protein A (NadA), factor H binding protein (fHbp), *Neisseria* heparin binding antigen (NHBA), and outer membrane vesicles (OMVs) from capsular group B *Neisseria meningitidis* strain NZ98/254—was developed, and for each antigen a reference strain is available to test immunogenicity using a serum bactericidal antibody (SBA) assay [2].

The licensed infant schedule consists of 3 primary doses between 2 and 6 months of age and 1 booster dose at 12 months [3–5]. The United Kingdom was the first country to implement 4CMenB in a national immunization schedule and to use a cost-effective reduced schedule of 2 primary doses at 2 and 4 months of age and 1 booster dose at 12 months of age [6]. The program started in September 2015, and a disease reduction of 50% (95% confidence interval [CI], 0.36–0.71; $P < .001$) was estimated, with an estimated vaccine effectiveness of 82.9% for all MenB cases [7].

Few studies have addressed the immunogenicity of the reduced dose schedule; here we describe immunogenicity, cellular immune responses (memory B cell), and the reactogenicity of a 2 + 1 schedule with the 4CMenB vaccine in UK infants.

METHODS

Study Design and Participants

In this single-center, randomized, open-label clinical trial performed in the United Kingdom, healthy Caucasian infants were recruited at the age of 8 to 12 weeks. Written informed consent

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from 1 of the parents or legal guardians was obtained at the time of the first visit.

In each study group (test vs control), the infants were divided into 4 subgroups (test group: SG1-4; control group: SG5-8), reflecting different visit timings, in order to optimize the collection of information and samples.

Study Objectives and End Points

The primary objective of this study was to describe the kinetics of global gene expression in whole blood after vaccination with 4CMenB vaccine in healthy infants.

This paper focuses on the description of the analysis of the secondary objectives: the immunogenicity of the 4CMenB 2 + 1 schedule after primary and booster vaccinations, the long-term immune responses after primary and booster immunizations with 4CMenB, and the reactogenicity profile of the 4CMenB vaccine.

Randomization and Procedures

Participants were randomly allocated to test (to receive 4CMenB vaccine at 2, 4, and 12 months of age, as per current UK schedule) or control (to receive 4CMenB at 6, 8, and 13 months of age) groups using sequentially numbered envelopes containing a concealed group allocation number.

All infants included in this study received immunizations according to the UK national immunization schedule implemented during the study period, including DTaP-IPV-Hib at 2, 3, and 4 months of age (PediaceL, Sanofi Pasteur); Rotavirus vaccine at 2 and 3 months of age (Rotarix, GlaxoSmithKline Biologicals); PCV13 at 2, 4, and 12 months of age (Prevenar-13, Pfizer); MenC-TT at 3 months (NeisVac-C, Baxter Vaccines); Hib-MenC-TT at 12 months of age (Mentorix, GSK); and MMR at 13 months of age (Priorix, GSK). All routine vaccines, except Rotarix, given orally, were administered in the antero-lateral left thigh at all time points.

A total of 6 blood samples were taken during the study period at specific time points both before and after vaccination.

Study Vaccine

4CMenB is an inactivated vaccine comprising 3 recombinant *Neisseria* proteins formulated with OMVs from serogroup B *N. meningitidis* strain NZ98/254, supplied in prefilled 1-mL syringes that deliver a single dose of 0.5 mL. Each 0.5 mL contains: 50 µg of NHBA, 50 µg of NadA; 50 µg of fHbp, 25 µg of OMV from *N. meningitidis* strain NZ98/254, and aluminum hydroxide adjuvant, NaCl, sucrose, histidine, and water for injection. The vaccine was administered intramuscularly in the antero-lateral right thigh.

Immunogenicity Evaluation

The immune response was measured in a human complement serum bactericidal antibody (hSBA) using the reference strain

44/76-SL, and titer ≥ 4 was considered putatively protective. The assay was performed by the Public Health England Vaccine Evaluation Unit (Manchester, UK) [8].

In the test group, hSBA was measured 4 weeks after primary and booster immunizations and in a subset of participants just before booster immunizations.

In the control group, the hSBA against the same reference strain was measured at 6 months of age (pre-primary immunizations), at 12 months of age (6 months post-primary immunizations), and 13 months of age (7 months post-primary immunizations).

Memory B-Cell ELISPOTs

Memory B-cell (Bmem cell) responses to specific proteins (fHbp, NadA, NHBA, and Porin A [PorA]) were analyzed using a cultured B-cell ELISpot assay. In the test group, the Bmem cell ELISpot was performed 4 weeks post-primary immunization, before the booster dose, and 4 weeks after the booster dose. In the control group, the Bmem cell was performed at baseline (6 months of age) and at 6 and 7 months after the second primary dose (12 and 13 months of age).

For the Bmem cell assay PVDF, 96-well plates (Millipore, Cork, Ireland) were coated with 100 µL of either 2.5 µg/mL of Por A, 1 µg/mL of NadA, 1 µg/mL of NHBA and 2 µg/mL of fHbp, or phosphate-buffered saline (as a negative/background control). The wells were blocked with complete medium (RPMI; 10% fetal bovine serum, penicillin/streptomycin, and L-glutamine, all from Sigma-Aldrich, St Louis, MO, USA) before the cells were seeded on the precoated plates.

Frozen peripheral blood mononuclear cells (PBMCs) previously isolated from fresh blood were cultured with complete medium plus *Staphylococcus aureus* Cowan strain at a 1:5000 dilution (VWR International Ltd, Lutterworth, UK), pokeweed mitogen at 83 ng/mL (Sigma-Aldrich, St Louis, MO, USA), and the CpG-2006 oligonucleotide at 1.7 µg/mL (Invivogen, San Diego, CA, USA). PBMCs were seeded at 2×10^5 cells/well and incubated for 6 days at 37°C in 5% carbon dioxide and 95% humidity. The final processing was performed according to Truck et al. [9].

Reactogenicity and Safety Analysis

To assess the vaccine reactogenicity, all local and systemic solicited and unsolicited reactions in the 7 days following the 2, 4, and 12 month immunizations were recorded. Axillary temperatures were measured by parents at least once a day in the first 7 days after immunization, except on the first day, when parents were requested to measure twice, at 4 and 8 hours postvaccination.

A skin sensor temperature monitoring device (iButton) with data logger, fitted on the surface of the infant abdominal wall, was issued for use in the first 24 hours after the 4- and 12-month immunizations, recording 1 temperature per minute.

The definition of fever was established as a temperature $\geq 38^{\circ}\text{C}$, according to the Brighton Collaboration Fever Working Group [10].

Severe adverse events (SAEs) were reported over the duration of the study.

Statistical Analysis

The secondary and exploratory objectives were analyzed on an intention-to-treat (ITT) basis. However, as 1 control group participant received their booster vaccination off-schedule, the hSBA analysis was also done on a per-protocol basis. The sample size calculation was performed considering only the primary end point.

A predefined correlate of protection of hSBA $\geq 1:4$ against reference strain 44/76SL was used. To calculate GMT (and 2-sided 95% CIs), hSBA titers were \log_{10} -transformed. For titers that were below the level of detection ($<1:2$), half of the value [1] was used.

The Bmem ELISpot data were captured using an automated ELISpot reader (AID Diagnostika GmbH, Strassberg, Germany). For the statistical analysis, Bmem cell frequency was adjusted to and expressed as antibody-secreting cells (ASCs)/ 1×10^6 PBMCs. To compare the data between time points, a Wilcoxon matched-pair signed-rank test was carried out.

The safety and reactogenicity analysis included all study participants who received at least 1 dose of the study vaccine.

Fever episodes, defined as a temperature $\geq 38^{\circ}\text{C}$, were analyzed at 4 and 12 months of age.

A descriptive analysis of temperatures was carried out on both the axillary temperatures and iButton data. To compare the iButton fever rates and temperatures between the groups, the chi-square test and 2-sided t test were used, respectively. Time to first fever was assessed using Kaplan-Meier failure curves and the log-rank test. Agreement between the iButton and axillary temperatures, measured at exact times (matched by minute), was assessed using Lin's concordance correlation coefficient and graphically represented by Bland-Altman plots (not presented).

The analyses were carried out using STATA/IC, version 14.2, and STATA/SE, version 15.1. A P value of .05 was considered statistically significant.

This trial was registered at ClinicalTrials.gov (NCT02080559).

RESULTS

This clinical trial was performed in the UK from July 2014 to June 2016, and a total of 187 infants were randomized to receive 4CMenB vaccine according to the schedule of UK routine immunizations at 2, 4, and 12 months of age (test group; $n = 94$) or 4CMenB alone at 6, 8, and 13 months (control group; $n = 93$). Ninety-two infants randomized to the test group and 89 from the control group completed the study (Figure 1, flow diagram).

The median age of infants at the time of enrollment (inter-quartile range) was 61 (58–66) days and 62 (60–66) days in the test and control groups, respectively. A higher percentage of boys were recruited in both groups (all: 54.6%; test group: 53.2%; control: 55.9%).

Protective SBA titers were induced by a primary course of 4CMenB and enhanced following a booster dose.

In the test group, 4 weeks after the 2 primary doses of 4CMenB at 2 and 4 months, 97.3% of the infants (95% CI, 90.5%–99.7%) had hSBA titers ≥ 4 against the reference strain 44/76-SL, showing putatively protective titers against this strain. A similar percentage of infants were also seropositive against the same strain (97.4%; 95% CI, 90.8%–99.7%) after the booster dose. The SBA GMT was higher in postbooster sera when compared with the postprimary time point (Table 1).

Waning of immunity was identified between the primary and booster immunizations, showing a decrease in the proportion of infants with sero-protective titers against the reference strain 44/76-SL (Figure 2).

4CMenB Induced a Bmem Response, Which Correlated With Postbooster SBA Titers

A significant increase in the frequency of Bmem cells for all specific antigens from postprimary (mean [SD], fHbp: 3.5 [4.6]; NadA: 5.5 [6.8]; NHBA: 5.0 [5.9]; PorA: 12.1 [12.6]) to postbooster (mean [SD], fHbp: 22.2 [37.3]; NadA: 20.3 [25.0]; NHBA: 10.8 [17.04]; PorA: 33.1 [41.0]) was found in the test group (figure 3). This was despite the test group having no difference in memory postpriming in comparison with the unprimed control group.

In the control group, for all specific antigens, Bmem cell frequencies significantly increased from baseline (mean [SD], fHbp: 11.1 [11.5]; NadA: 11.1 [9.5]; NHBA: 14.7 [11.8]; PorA: 18.9 [14.5]) after 2 late primary doses and at 12 (mean [SD], fHbp: 32.9 [22.6]; NadA: 25.5 [20.9]; NHBA: 26.9 [22.5]; PorA: 30.1 [17.4]) and 13 months of age (mean [SD], fHbp: 28.3 [25.3]; NadA: 27.2 [15.1]; NHBA: 23.7 [18.5]; PorA: 25.1 [18.9]) (Figure 3).

A Spearman correlation of .44 ($P = .004$) was identified between the postbooster hSBA and the fHbp-specific Bmem cells performed 1 month after primary immunization in the test group.

Reactogenicity and Safety Profiles Were Similar in the Test and Control Groups

In general, the test group had a higher number of local reactions when compared with the control group, particularly after the 2- and 12-month immunizations, but no statistically significant differences were found between the groups. The 4CMenB vaccine caused a higher number of local reactions when compared with routine immunizations in the same group.

Change in eating habits, drowsiness, and irritability/crying were more common in the test group when compared with the control group for all time points (table 2). In both groups,

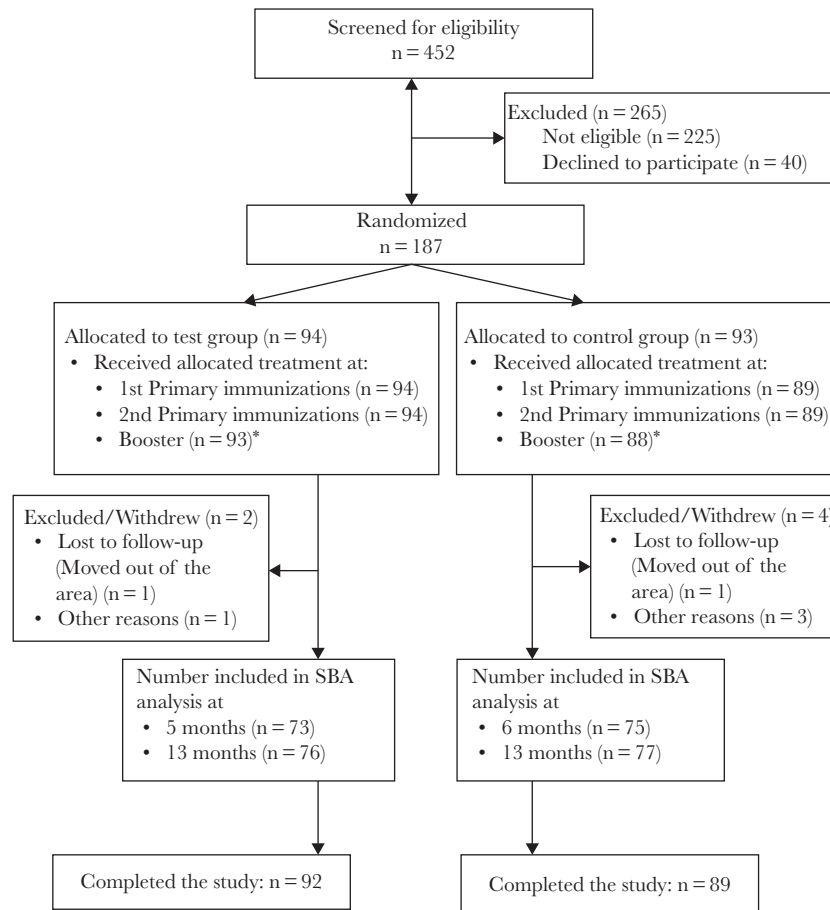


Figure 1. Clinical trial flow diagram (CONSORT diagram). Children were considered not eligible for the study if they failed to fulfill all inclusion criteria (healthy infants of 2 Caucasian parents, born between 37 and 42 weeks of gestation and aged 8–12 weeks at the time of first visit; parent or legal guardian willing and able to comply with the requirements of the protocol, who had Internet access for the duration of the study; parent/legal guardian who had given informed consent for their child’s participation in the study) or if they had any exclusion criteria (infants with a history of invasive MenB disease, previous vaccination with an MenB vaccine, a household contact with a case of confirmed bacterial meningitis, confirmed or suspected immunodeficiency, family history of congenital or hereditary immunodeficiency or maternal HIV, in receipt of >1 week of immunosuppressants or immune-modifying drugs, a history of allergy to a component of the vaccine, major congenital defects or serious chronic illness, history of neurologic disorders, or administration of immunoglobulins or any blood products since birth). Abbreviation: SBA, serum bactericidal antibody. *One control group participant received routine and 4CMenB vaccines at the 12-month visit (V10) rather than at the 13-month visit (V12).

Table 1. Strain 44/76-SL hSBA Results: Proportion of Individuals With hSBA ≥ 4 and Geometric Mean Titers, by Visit and Group

Age	Group	No.	hSBA Titer ≥ 4		SBA GMT (95% CI)
			No. (%)	95% CI	
5 mo	Test	73	71 (97.3)	90.5–99.7	48.6 (37.2–63.4)
6 mo	Control	74	21 (28.4)	18.5–40.1	2.1 (1.7–2.4)
12 mo	Test	48	14 (29.2)	17.0–44.1	2.2 (1.8–2.8)
13 mo	Test	76	74 (97.4)	90.8–99.7	72.1 (51.7–100.4)
	Control	77	47 (61.0)	49.2–72.0	4.7 (3.7–6.1)

Abbreviations: CI, confidence interval; hSBA, human complement serum bactericidal antibody; SBA, serum bactericidal antibody.

irritability was the most common systemic event reported, and differences between groups were considered statistically significant for the 12-month immunizations ($P = .001$).

From the 187 infants enrolled, iButton records were available for 177 infants at 4 months and 174 at 12 months of age.

At 4 and 12 months of age, the rates of fever were higher in the test group (4CMenB + routine immunization groups) regardless of the technique used (4 months: iButton: 56.4%; axillary temperature measurement: 12.8%; 12 months: iButton: 56.4%; axillary temperature measurement: 10.6%) when compared with control group (4 months: iButton: 25.8%; axillary temperature measurement: 6.5%; 12 months: iButton: 25.8%; axillary temperature measurement: 7.5%). At both time points, the rates of fever (at least 1 fever episode) were significantly higher ($P < .001$) in the test group than the control group.

In those who experienced a fever, 50% developed a temperature at around 5 hours after immunization in the test and control groups at 4 and 12 months of age, according to the iButton.

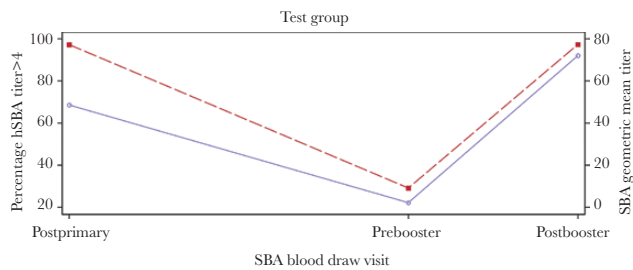


Figure 2. Proportion of individuals with human complement serum bactericidal antibody titers ≥ 4 after postprimary, prebooster, and postbooster immunizations in the test group. Abbreviation: SBA, serum bactericidal antibody.

Although statistically significant, the agreement between axillary temperature and iButton was considered weak.

A total of 13 SAEs (all unrelated to the study vaccine) were reported, of which 6 were in the test group and 7 were in the control group. The most common diagnosis was bronchiolitis [4], followed by nonspecified acute respiratory illness with wheezing [3], abscess [2], unspecified viral illness [1], hypothermia [1], cow milk protein intolerance/allergy [1], fever with unknown origin with negative bacterial culture [1], and varicella with secondary bacterial infection [1].

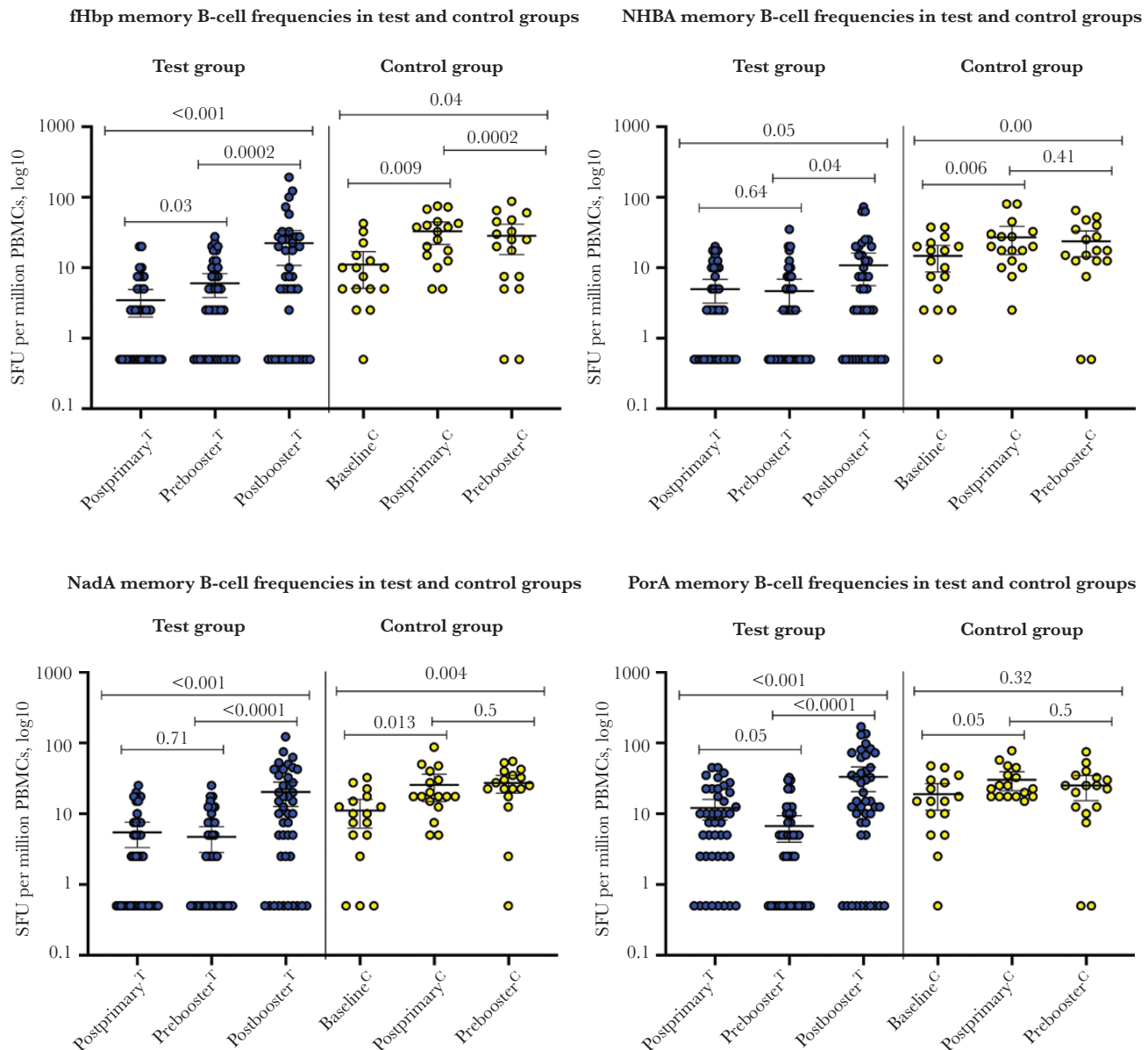


Figure 3. Dot plots of antigen-specific memory B-cells after ELISpot assay for the test group and control group at different time points (mean and 95% confidence interval). Test group: Postprimary^T: 1 month after primary immunizations – 5 months of age; Prebooster^T: 8 months after primary immunizations – 12 months of age; Postbooster: 1 month after booster immunizations – 13 months of age. Control group: Baseline^C: 6 months of age; Postprimary^C: 4 months after primary immunizations – 12 months of age; Prebooster^C: 5 months after primary immunizations – 13 months of age. Abbreviations: NHBA, *Neisseria* heparin binding antigen; PBMC, peripheral blood mononuclear cell.

Table 2. Number of Participants With at Least 1 Solicited Reaction in the 7 Days After Immunization at 2, 4, and 12 Months of Age

		Solicited Reactions		
Diary	Reaction	Control, No. (%)	Test, No. (%)	PValue*
2 mo of age, No. (%)		(n = 88)	(n = 93)	
	Change in eating habit	31 (34.8)	56 (59.6)	.001
	Drowsiness	52 (58.4)	61 (64.9)	.368
	Irritability/fussiness	76 (85.4)	85 (90.4)	.295
4 mo of age, No. (%)				
	Change in eating habit	25 (28.1)	36 (38.7)	.129
	Drowsiness	47 (52.8)	66 (71.0)	.012
	Irritability/fussiness	65 (73.0)	74 (79.6)	.299
12 mo of age, No. (%)				
	Change in eating habit	33 (37.5)	52 (55.9)	.013
	Drowsiness	35 (39.8)	55 (59.1)	.009
	Irritability/fussiness	49 (55.7)	73 (78.5)	.001

*Based on the chi-square test.

The odds ratio for the comparison in the test group was 0.84 (95% CI, 0.2–3.05), indicating no statistically significant association between concomitant 4CMenB and the occurrence of SAEs.

DISCUSSION

In this study, the 4CMenB reduced schedule (2 + 1), when given concomitantly with the other routine vaccines included in the UK infant immunization program, induced serum bactericidal antibody titers above the putatively protective threshold against the reference strain 44/76-SL after primary and booster doses. With more than 97% of the infants having a putative protective hSBA titer (hSBA ≥ 4), these proportions with evidence of seroprotection were similar to the ones reported by previous studies, when using the licensed schedule with 3 primary doses [3, 5], or 1 earlier study using a reduced schedule [11]. Assuming that the proposed threshold does correlate with protection, these data are in concordance with the effectiveness studies done in the UK after the implementation of the reduced schedule [7].

Although serological evidence of protection was achieved in the majority of the vaccinated study individuals, the GMTs 4 weeks after primary (48.6; 95% CI, 37.2–63.4) and booster doses (72.1; 95% CI, 51.7–100.4) for the same reference strain were lower than those reported in previous studies using the full licensed schedule (3 + 1, Vesikari et al. [3]; postprimary GMT: 91; 95% CI, 88–95; postbooster GMT: 139; 95% CI, 124–156; and Gossger et al. [5]; postprimary GMT: 83; 95% CI, 77–90). The sero-response was also lower when compared with a previous study that also used a reduced schedule [11], although the 4CMenB was given at an older age in that trial (3 and a half months and 5 months of age).

The clinical importance of a lower GMT is uncertain, particularly in the context of documented effectiveness in the UK

population. Waning of protection might be more rapid with a lower initial immune response, but thus far this has not been reported as an issue in the United Kingdom. For a subset of individuals in the test group, prebooster hSBA (8 months after the last primary dose) was determined, showing a decrease in seroprotection to 29.2%, with a GMT of just 2.2 (95% CI, 1.8–2.8). The waning of immunity was less marked in a study by Vesikari et al. [3], with a loss of protection before booster of ~20% and a GMT of 11.

In our study, the availability of serum, after the blood for the primary end point was prioritized, was low, so hSBA was only determined against 1 reference strain (44/56SL). The strain used tested immunogenicity against fHbp [12]. This strain was selected because of the importance of its antigenic composition as a target for the 4CMenB component [12].

Memory B-cell responses were assessed at different time points after immunization, but a specific Bmem cell response was not detectable after the primary immunizations, perhaps due to the low frequency of cells induced by the vaccine, below the level of detection in this assay [13, 14]. Similarly, Kelly et al., in an infant study analyzing the kinetics of capsular group C (MenC) meningococcal Bmem cells, showed that MenC-specific Bmem cells were very low to undetectable 4 weeks after a single primary dose of MenC conjugate vaccine [14]. However, in the same study, MenC Bmem frequency increased significantly after a third dose of the vaccine, but there were no data following the second primary dose [15]. Prebooster-specific Bmem cells against 6 pneumococcal serotype frequencies were determined in an infant population, showing low levels 8 months after primary immunizations [9]. Those frequencies increased significantly after the booster dose, similar to the results in our study [9]. Although in this specific study we did not measure the persistence of antibodies after the booster dose, previous studies have shown a waning of immunity after the booster dose in children at different ages [16–18]. Independent of the significant increase of Bmem cells after the booster dose found in this study, duration and persistence of immunity after the 4CMenB vaccine, as shown in the previously described studies, seem to be limited in time.

A correlation between Bmem cells and immunogenicity has been described, with different results depending on the vaccine, number of doses, and age of the individuals [9, 14, 19]. We found a weak but significant correlation (Spearman $\rho = .44$; $P = .004$) between the postbooster hSBA and the fHbp-specific Bmem cells measured at 1 month after primary immunizations in the test group.

Consistent with previous work, the use of 4CMenB was safe, with no SAEs reported to be related to the study vaccine administration.

As reported in previous studies and described in this study, the administration of 4CMenB concomitantly with other routine vaccines increases reactogenicity. Currently in the United

Kingdom, 3 doses of paracetamol are recommended at the 2- and 4-month immunizations. In this study, paracetamol was only given if required, as at that time of the study, the 4CMenB vaccine had not been included in the UK schedule.

The use of the iButton confirmed higher fever rates in the test group when compared with the control group. Rates of fever detected with the iButton were much higher than those measured with standard intermittent axillary temperature collection. The Brighton collaboration guidelines define that temperatures should be measured at specific timings, adapted to the vaccine biological activity. Our study shows that the use of intermittent methods could potentially underestimate the number of fever episodes, and a continuous method could potentially allow a more accurate determination of fever rates.

Our study had some limitations, including limited availability of serum for the assessment of immunogenicity; thus only 1 reference strain (44/56SL) was selected because of the importance of its antigenic composition as a target for the 4CMenB component fHbp [12].

In this clinical trial, we have shown that a reduced infant 4CMenB schedule is safe, immunogenic, and induces detectable immune memory after the booster dose.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. M.V.P. is a member of the Portuguese National Immunisation Technical Advisory Group (Comissão Técnica de Vacinação da Direção Geral de Saúde). A.J.P. chairs the UK Department of Health's Joint Committee on Vaccination and Immunisation and the EMA Scientific Advisory Group on vaccines, and he is a member of the WHO's Strategic Advisory Group of Experts. M.D.S. acts on behalf of the University of Oxford and Oxford Vaccine Group (OVG) as Chief or Principal Investigator on clinical trials sponsored and/or funded by vaccine manufacturers including Pfizer and GSK. J.P., M.V.P., and E.P. are employed by the OVG. M.D.S. has participated in advisory boards for vaccine manufacturers and in industry-sponsored symposia. Payment for these

activities was made to the University of Oxford, and M.D.S. received no personal financial benefits. R.B. performs contract research on behalf of Public Health England for GSK, Pfizer, and Sanofi Pasteur. At the time of the study, H.F. was an employee of PHE and performed contract research on behalf of PHE for GSK and Sanofi Pasteur. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. A.J.P. and D.O.C. designed the trial. M.V.P., M.D.S., E.P., H.R., S.K., Y.F.M., and A.J.P. oversaw the clinical trial, clinical data collection, and clinical data management. U.G. and M.V.P. conducted the statistical analysis. E.A.C., S.B., S.C.P., H.H., H.F., and R.B. conducted the laboratory assays. M.V.P. wrote the first draft of the paper, and all authors contributed to subsequent drafts. All read and approved the final version of the report.

Ethical approval. This study received ethical approval from the Oxfordshire Research Ethics Committee (reference number: 14/SC/0077) and is registered on the EudraCT clinical trials database (2014-000126-38) and at ClinicalTrials.gov (NCT02080559).

Prior presentations. 37th Annual Meeting of ESPID, Ljubljana, Slovenia, 2019 (abstract 19-0491)—presentation of preliminary data on memory B cells.

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