



Safety and immunogenicity of a pentavalent meningococcal conjugate vaccine containing serogroups A, C, Y, W, and X in healthy adults: a phase 1, single-centre, double-blind, randomised, controlled study

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Summary

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Background Invasive meningococcal disease is an important public health problem, especially in sub-Saharan Africa. After introduction of MenAfriVac in 2010, *Neisseria meningitidis* serogroup A disease has been almost eliminated from the region. However, serogroups C, W, Y, and X continue to cause disease outbreaks. We assessed the NmCV-5 pentavalent meningococcal conjugate vaccine targeting A, C, Y, W, and X serogroups in a first-in-man, phase 1 study.

Methods We did a single-centre, double-blind, randomised controlled trial at a research clinic in Baltimore (MD, USA). Participants were healthy adults aged 18–45 years with no history of meningococcal vaccination or previous meningococcal infection. We randomly assigned participants (1:1:1) by an SAS-generated random schedule to a single, 0·5 mL, intramuscular injection of aluminium-phosphate adjuvanted NmCV-5, non-adjuvanted NmCV-5, or control (the quadrivalent meningococcal conjugate vaccine Menactra). The randomisation sequence used a permuted block design with randomly chosen block sizes of three and six. The vaccines were prepared, labelled, and administered with procedures to ensure participants and study personnel remained masked to treatment. After vaccination, participants were observed in the clinic for 60 min for adverse reactions. Participants recorded daily temperature and injection site or systemic reactions at home and returned to the clinic for follow-up visits on days 7, 28, and 84 for safety assessments; blood samples were also collected on day 7 for safety laboratory assessment. A phone call contact was made 6 months after vaccination. Serum was collected before vaccination and 28 days after vaccination for immunological assessment with a rabbit complement-dependent serum bactericidal antibody (rSBA) assay. The primary objective was an intention-to-treat assessment of safety, measuring local and systemic reactogenicity over 7 days, unsolicited adverse events through 28 days, and serious adverse events over 6 months. The secondary objective for the assessment of immunogenicity, was a per-protocol analysis of rSBA before and 28 days after vaccination. This trial is registered with ClinicalTrials.gov, number NCT02810340.

Findings Between Aug 17, 2016, and Feb 16, 2017, we assigned 20 participants to each vaccine. All vaccines were well-tolerated. Pain was the most common local reaction, occurring in 12 (60%), ten (50%), and seven (35%) participants in the adjuvanted NmCV-5, non-adjuvanted NmCV-5, and control groups, respectively. Headache was the most common systemic reaction, occurring in five (25%), three (15%), and three (15%), respectively. Most solicited reactogenicity adverse reactions were mild (60 [74%] of 81) and all were self-limiting. None of the differences in proportions of individuals with each solicited reaction was significant ($p > 0\cdot300$ for all comparisons) between the three vaccination groups. There were no serious adverse events and 19 unsolicited non-serious adverse events in 14 (23%) participants. Both adjuvanted and non-adjuvanted NmCV-5 elicited high rSBA titres against all five meningococcal serogroups. The pre-vaccination geometric mean titres (GMTs) ranged from 3·36 to 53·80 for the control, from 6·28 to 187·00 for the adjuvanted vaccine, and from 4·29 to 350·00 for the non-adjuvanted vaccine, and the post-vaccination GMT ranged from 3·14 to 3214 for the control, from 1351 to 8192 for the adjuvanted vaccine, and from 1607 to 11191 for the non-adjuvanted vaccine. Predicted seroprotective responses (ie, an increase in rSBA titres of eight times or more) for the adjuvanted and non-adjuvanted NmCV-5 were similar to control responses for all five serogroups.

Interpretation The adjuvanted and non-adjuvanted NmCV-5 vaccines were well tolerated and did not produce concerning adverse effects and resulted in immune responses that are predicted to confer protection against all five targeted serogroups of invasive meningococcal disease. Further clinical testing of NmCV-5 is ongoing, and additional clinical trials are necessary to confirm the safety and immunogenicity of NmCV-5 in target populations.

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Research in context

Evidence before this study

Despite the success of MenAfriVac vaccine, invasive meningococcal disease caused by *Neisseria meningitidis* serogroups C, W, Y, and X continues to cause substantial outbreaks in the African meningitis belt. We searched PubMed on May 7, 2018, without language restrictions, for studies with the following search terms in the title or abstract: (“Meningococcal Conjugate Vaccine” OR “Meningitis Vaccine”) AND (“Serogroup X”), restricting the article type to clinical trials and the species to human. This search identified no relevant articles. We then relaxed the search terms by removing the clinical trial article type and identified 40 publications, none of which were relevant to the clinical assessment of a meningococcal vaccine containing serogroup X.

Added value of this study

NmCV-5 is a pentavalent meningococcal conjugate vaccine targeting A, C, W, Y, and X serogroups that was developed to further address invasive meningococcal disease. The vaccine was manufactured using the same facility and same strain of serogroup A (M1027) as that of MenAfriVac.

This first-in-human study showed that there were few safety concerns and significant serum bactericidal antibody responses against all five serogroups with both aluminium-phosphate adjuvanted or non-adjuvanted NmCV-5.

Implications of all the available evidence

The currently available licensed multivalent meningococcal conjugate vaccines do not provide protection against serogroup X meningococcal disease and are not affordable for wide-scale implementation in low-resource countries, such as those in the African meningitis belt. NmCV-5, in our phase 1 trial, was well tolerated and elicited serum antibodies that are predicted to confer protection against all five serogroups. Additional clinical trials are necessary to confirm the safety and immunogenicity of NmCV-5 in target populations. Ultimately, NmCV-5 could be introduced into sub-Saharan Africa for further elimination of meningococcal disease. Outside the meningitis belt, NmCV-5 could also be used in other endemic countries, for travellers to sub-Saharan Africa, and during Hajj or Umrah.

Introduction

Invasive meningococcal disease is a formidable public health concern because of its rapid clinical onset, its predilection for causing high rates of death or permanent disability, and the inability to accurately forecast when an epidemic or sporadic cases might occur. Most of the global burden of meningococcal disease is located in a band of countries within sub-Saharan Africa.¹ This so-called meningitis belt, which stretches from Senegal to Ethiopia, might have an annual incidence of meningococcal disease of 100 cases per 100 000 individuals, with epidemic peaks up to 1000 cases per 100 000 individuals.² One of the worst documented meningococcal epidemics on record occurred during 1996–97 and is estimated to have resulted in more than 250 000 cases, 25 000 deaths, and 50 000 disabilities.³

The Meningitis Vaccine Project, created in 2001, and representing a partnership between WHO and the Program for Appropriate Technology in Health (now known as PATH) developed MenAfriVac, a *Neisseria meningitidis* serogroup A polysaccharide conjugated to tetanus toxoid vaccine (Serum Institute of India Pvt, Pune, India).⁴ Before the introduction of MenAfriVac in 2010, *N meningitidis* serogroup A was responsible for most of the meningococcal disease (about 70%–96%) in sub-Saharan Africa.⁵ The progressive introduction of MenAfriVac brought about a substantial reduction in cases of serogroup A meningococcal disease, such that by 2011–13, the incidence had dropped to 0·02 cases per 100 000,⁶ and during 2017, only two cases of serogroup A disease were detected.⁷ Thus, the invasive meningococcal disease landscape in the period after the introduction of MenAfriVac is characterised by non-serogroup A disease.⁸

Sporadic epidemics of non-serogroup A meningococcal disease have been documented in sub-Saharan Africa. Serogroup W (formerly W-135) was previously associated with infrequent epidemics, but an *N meningitidis* serogroup W clone (ST11/ET37) was the cause of a substantial outbreak during the Hajj in 2000,⁹ and serogroup W has since been a major cause of meningococcal disease in sub-Saharan Africa.¹⁰ *N meningitidis* serogroup X is another important cause of meningococcal disease in sub-Saharan Africa, responsible for outbreaks in Niger, Uganda, Kenya, Togo, and Burkina Faso from 2006 through 2010.¹¹ Although serogroup C epidemics had occurred in the 1970s—eg, in 1979 in Upper Volta (present-day Burkina Faso)¹²—since 2013 a novel *N meningitidis* serogroup C clone (ST10217)¹³ has emerged, resulting in a marked rise in the burden of serogroup C disease in sub-Saharan Africa.^{14,15} From January through June, 2015, more than 8000 cases and 500 deaths were associated with serogroup C infections in Niger alone.¹⁶ In 2017, serogroup C was predominant, comprising 31·8% of positive samples, followed by serogroup X (11·9%) and serogroup W (9·4%).⁷ Disease caused by serogroups Y and B is rare in sub-Saharan Africa; nonetheless, outbreaks for many of these serogroups have been reported in other regions in the world.¹⁷

Currently licensed multivalent meningococcal conjugate vaccines do not provide protection against serogroup X disease and are also not affordable in low-resource countries. Building upon the striking success of MenAfriVac and to further move toward the potential elimination of meningococcal disease in sub-Saharan Africa, the NmCV-5 combined pentavalent meningococcal

polysaccharide conjugate vaccine is being developed. The successful process development and positive immunogenicity in mice of a monovalent, serogroup X polysaccharide, tetanus-toxoid conjugate vaccine suggested that an efficacious and cost-effective vaccine against serogroup X for human beings was possible.¹⁸

Here, we report the first-in-man assessment of the NmCV-5 vaccine administered in a single intramuscular dose with or without aluminium phosphate adjuvant. The primary objective was the assessment of the safety of the study vaccine, with and without adjuvant; the hypothesis was that the two vaccine formulations would be well tolerated. The secondary objective was the measurement of the immune response to the study vaccine, with and without adjuvant; the hypothesis was that the two vaccine formulations would induce immune responses to all five serogroups included in the vaccines.

Methods

Study design and participants

We did a single-centre, phase 1, double-blind, randomised, controlled trial at a research clinic in Baltimore (MD, USA). Eligible participants were healthy adults aged 18–45 years with no history of meningococcal vaccination or previous meningococcal infection. Individuals with any chronic medical conditions, immunodeficiencies, or pregnancy noted during screening were excluded. The complete eligibility criteria are in the appendix. The study was approved by the University of Maryland Baltimore Institutional Review Board. All study participants gave written informed consent before the initiation of any study procedures.

See Online for appendix

Randomisation and masking

We randomly assigned participants (1:1:1) to non-adjuvanted NmCV-5 vaccine, adjuvanted NmCV-5 vaccine, or control (the licensed quadrivalent conjugate vaccine Menactra). On the day of enrolment, demographic and eligibility information was entered by masked staff into an electronic data system. The randomisation sequence was generated by the trial statistician in SAS (version 9.3) using a permuted block design with randomly chosen block sizes of three and six. Upon completion of entry of a participant's enrolment information, a concealed treatment assignment number was provided in the electronic data entry system. The number was used by the unmasked research pharmacist to prepare the allocated product for each participant, using a treatment code list that was maintained by the pharmacist in a secure location, to maintain blinding. The vaccines were filled into identical-appearing syringes and labelled in a manner such that each syringe could be matched to the study participant but did not provide unblinding information, and they were administered with procedures to ensure participants and prospectively assigned study personnel remained masked to treatment.

Procedures

Participants were given a single 0.5 mL intramuscular injection of one of adjuvanted NmCV-5, non-adjuvanted NmCV-5, or Menactra. NmCV-5 was manufactured by the Serum Institute of India Pvt Ltd (Pune, India) and consists of *N meningitidis* serogroups A and X polysaccharides individually conjugated to tetanus toxoid and poly-saccharides for serogroups C, Y, and W individually conjugated to recombinant CRM₁₉₇ protein. The strains for serogroups A, C, and W were received from the Center for Biologics Evaluation and Research, US Food and Drug Administration, and the serogroup Y and X strains were received from the US Centers for Disease Control and Prevention. The processes for production of serogroup A, C, Y, and W polysaccharides are well established through fed batch fermentation processes.^{19,20} The polysaccharide purification protocols were largely based on surfactant precipitation of polysaccharide and alcohol extraction. Accordingly, the polysaccharides for serogroups A, C, W and Y were O-acetylated. Previously reported fermentation media did not support the growth of serogroup X; therefore, a fermentation medium was developed that resulted in acceptable yields of purified serogroup X polysaccharide.¹⁸ The sizing methods resulted in polysaccharides with desired polydispersity indices (1 [SD 0.3] for all polysaccharides), thus further fractionation was not necessary and diafiltration using 10 kDa cassettes was done before conjugation. Polysaccharides for serogroups A and X were size-reduced, activated by 1-cyano-4-pyrrolidinopyridinium tetrafluoroborate (CPPT), derivatised by adipic acid dihydrazide (ADH), and covalently coupled to chromatographically purified tetanus toxoid by carbodiimide chemistry.²¹ The size-reduced polysaccharides for serogroups C, Y, and W were activated by CPPT and conjugated to ADH-derivatised recombinant CRM197 carrier protein, using carbodiimide chemistry. The five monovalent conjugates (drug substances) were mixed to achieve the required final concentrations followed by the addition of stabilisers (ie, sucrose, sodium citrate, and trometamol).²² The final formulated bulk was filled into clear, type-1 glass vials (US Pharmacopeia) and lyophilised.²³

NmCV-5 was provided as a lyophilised powder to be reconstituted with 0.9% sodium chloride (non-adjuvanted vaccine) or with 125 µg aluminium phosphate in 0.9% sodium chloride (adjuvanted vaccine). A single intramuscular dose of 0.5 mL of vaccine (adjuvanted and non-adjuvanted) contains 5 µg of each of the five conjugated polysaccharides. The control vaccine was a single lot of commercially acquired Menactra vaccine (Sanofi Pasteur, Swiftwater, PA, USA), for which a single 0.5 mL intramuscular dose contains 4 µg each of polysaccharides of serogroups A, C, Y, and W conjugated to diphtheria toxoid without adjuvant. All study products were prepared by the Investigational Drug Service Pharmacy,

University of Maryland Medical Center (MD, USA) and administered by a prospectively assigned unmasked vaccinator.

After receiving the vaccination, participants were observed for 60 min in the clinic for systemic reactions (fever, headache, malaise, myalgia, arthralgia, diarrhoea, and anorexia) and local injection-site reactions (pain, erythema, and induration). For the following 7 days, participants recorded daily oral temperature and the occurrence of any injection site or systemic reactions at home. Participants graded the severity of their symptoms as mild (no interference with normal activities), moderate (some interference with normal activities), or severe (prevention of normal activities). Study participants returned for a clinic visit on days 7, 28, and 84 after vaccination; a phone call contact was made 6 months after vaccination. All recording of solicited reactogenicity and unsolicited adverse events was done by prospectively assigned, masked personnel, who remained masked for the duration of the study. Blood was collected 7 days after vaccination for safety laboratory assessments that consisted of measuring white blood-cell count and concentrations of haemoglobin, platelets, alanine aminotransferase (ALT), creatinine, albumin, and total bilirubin. Clinical signs and abnormal laboratory values were graded using predefined criteria. The occurrence of adverse events was recorded through 28 days after vaccination, and serious adverse events were recorded through 180 days after vaccination.

The measurement of functional antibodies by a rabbit complement-dependent serum bactericidal antibody (rSBA) assay is a surrogate of a meningococcal conjugate vaccine's ability to elicit serogroup-specific protection.^{24,25} Serum was collected before vaccination and 28 days after vaccination for immunological assessment. The per-protocol definition was any participant who was vaccinated and for whom post-vaccination immunogenicity data were obtained. The Vaccine Evaluation Unit at Public Health England (PHE, Manchester, UK) measured the rSBA titres against five target strains, each expressing the relevant serogroup-specific polysaccharide. The PHE rSBA assays for serogroups A and C are internationally standardised assays;²⁶ the rSBA assay method was adapted for serogroups Y, W, and X, as previously published.^{27–29} The target strains in the rSBA assays were: serogroup A F8238, serogroup C C11, serogroup Y S1975, serogroup W M01 240070, and serogroup X BF2/97.²⁷ Increases in serogroup C rSBA titres of eight times or more are deemed as an appropriate conservative threshold of protection in seronegative individuals,³⁰ and an increase in rSBA titres of 128 times or more has been proposed as an appropriate threshold for correlate of protection in seropositive individuals.³¹ Thus we used rSBA titre thresholds of eight times or more and 128 times or more in our analysis.

Outcomes

The primary objective was the assessment of safety with an intention-to-treat analysis of local and systemic

reactogenicity over 7 days after vaccination, and measurement of unsolicited adverse events through 28 days, and serious adverse events over 6 months. The secondary objective was the assessment of immunogenicity with a per-protocol analysis of rSBA titres at baseline (before vaccination) and 28 days after vaccination.

Statistical analysis

Although we did not do a formal sample size calculation, we selected the number of participants as deemed to be appropriate for a first-in-man study and the objectives were aimed to descriptively assess the safety and immunogenicity profile of the study products. The study design allowed a greater than 90% chance of observing an adverse effect that had an 11% chance of occurrence. Conversely, if no adverse effects occurred, the study was designed to be able to rule out events occurring in approximately 14% of the participants, based on the upper bounds of the one-sided 95% CI. We summarised local and systemic reactogenicity symptoms using the number and percentage of participants who had each event overall and compared the three treatment groups by Fisher's exact two-tailed test. The immunogenicity objectives were to measure the following: the proportion of participants with seroconversion, defined by an increase of four times or more in rSBA titre to each serogroup; the proportion of participants with seroprotective titres (or titres that were likely to predict protection), as defined using the two cutoff values of eight or more and 128 or more after vaccination for each serogroup; and rSBA geometric mean titres (GMT) for each serogroup. GMTs were computed by transforming the results to a logarithmic scale to satisfy asymptotic normality conditions, computing the mean, then converting the mean value back to the original scale. Comparisons in proportions were based on the unconditional exact 95% CI for treatment-group differences,³² where statistical significance at the 5% level was indicated by a CI that was entirely below or above 0. Comparisons in GMTs were based on the 95% CIs for the difference in treatment-group mean log titres, which, when back-transformed, were presented as ratios on the original scale; statistical significance at the 5% level was indicated by a CI that was entirely below or above one (unity). All data analyses and statistical computations were done with SAS software, version 9.3. This study was overseen by a safety review committee, and is registered with ClinicalTrials.gov, number NCT02810340.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 17, 2016, and Feb 16, 2017, we enrolled 60 eligible participants to the study and assigned 20 to

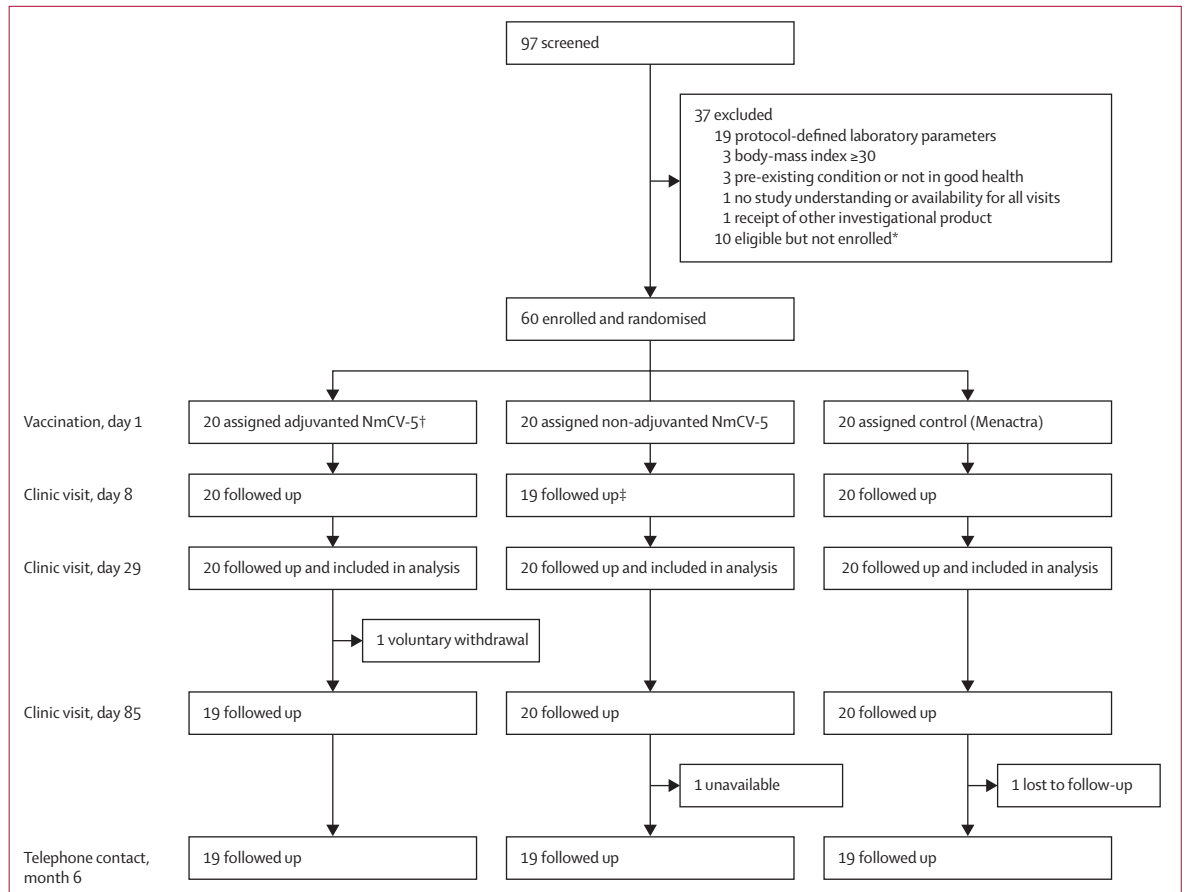


Figure: Trial profile

*Ten individuals were eligible at screening but were not enrolled for the following reasons: three could not commit the time; three were unable to be contacted; two screening window expired; one unsatisfied with study payment; and one self-report of influenza. †Aluminium-phosphate adjuvanted NmCV-5 vaccine. ‡One participant was unavailable for the day 8 clinic visit but was available for the follow-up visits on days 29 and 85.

	Total (n=60)	Adjuvanted NmCV-5* (n=20)	Non-adjuvanted NmCV-5 (n=20)	Menactra† (control; n=20)
Age (years)				
Mean (SD)	32.5 (7.07)	33.9 (6.38)	30.4 (7.23)	33.1 (7.43)
Range	18–45	23–45	18–45	20–43
Race				
Black	33 (55%)	11 (55%)	9 (45%)	13 (65%)
White	21 (35%)	5 (25%)	10 (50%)	6 (30%)
Asian	3 (5%)	1 (5%)	1 (5%)	1 (5%)
Native American or Alaskan Native	1 (2%)	1 (5%)	0	0
Mixed race	2 (3%)	2 (10%)	0	0
Ethnicity				
Hispanic	1 (2%)	0	1 (5%)	0
Non-Hispanic	59 (98%)	20 (100%)	19 (95%)	20 (100%)

*Aluminium-phosphate adjuvanted NmCV-5 vaccine. †Quadrivalent meningococcal conjugate vaccine Menactra (MCV4).

Table 1: Baseline characteristics of the intention-to-treat population

each vaccine (figure). Sex, race, and ethnicity were well distributed (table 1). There was no overt imbalance in the randomisation of participants to the three groups. All 60 randomly assigned participants were part of the full analysis population and completed the 28-day post-vaccination visit; thus there were no missing immunogenicity data and no imputation was required.

During the first 7 days after vaccination, there were no severe or potentially life-threatening solicited systemic or local reactions (table 2). Pain was the most common local injection site reaction; the most common systemic reactions recorded were headache, myalgia, and malaise. Of 81 solicited reactogenicity events recorded, most were mild (61; 75%) and a few were of moderate severity (20; 25%); all reactions were self-limited.

Of participants with any local or systemic reaction, five (25%) in the adjuvanted group, five (25%) in the non-adjuvanted group, and two (10%) in the control group had an event of moderate severity. 13 (65%) of 20, 12 (60%) of 20, and ten (50%) of 20 had at least one solicited event in the adjuvanted, non-adjuvanted, and control groups,

respectively. The proportions with at least one solicited systemic reaction were the same (eight [40%]) in all three groups and the proportions with any local reaction were 12 (60%), ten (50%), and eight (40%), respectively. None of the differences in proportions of participants with each solicited reaction was significant ($p > 0.300$ for all comparisons) between the three vaccination groups.

There were no serious adverse events and 19 unsolicited non-serious adverse events experienced by 14 participants (23%; appendix). The unsolicited adverse events were categorised into 13 MedDRA preferred term events: one graded as severe (toothache, in one non-adjuvanted NmCV-5 recipient) and six were moderate (upper respiratory infection in one non-adjuvanted NmCV-5 recipient; one each of bacterial vaginosis, viral illness, and toothache in adjuvanted NmCV-5 recipients; and one each of myalgia and hyperbilirubinaemia in control recipients). None of these events were deemed related to vaccination and all resolved without sequelae. There were 24 abnormal safety laboratory measures; all were of mild severity, except for a single moderate increase in bilirubin (to 1.6–2.5 times the upper limit of normal) in the control group. There was no trend for excessive reactivity, unsolicited adverse events, or laboratory abnormalities in any of the vaccination groups. A single pregnancy occurred in a participant who had received the control vaccine; the pregnancy had a normal outcome.

At least 70% of participants showed seroconversion (ie, an increase in rSBA titre of four times or more) for all five serogroups in the two NmCV-5 vaccine groups and in the control group for all serogroups except X. As expected, few participants in the control Menactra group (which does not include serogroup X) showed seroconversion for serogroup X (one [5%] of 20). The proportions of participants who achieved seroconversion against serogroups A, C, Y, and W were higher in both the adjuvanted and non-adjuvanted NmCV-5 groups, compared with those in the control vaccine group, although significance was reached only for serogroup W for which 20% more participants achieved seroconversion in the adjuvanted NmCV-5 group than in the control group (95% CI for the difference 1.4–43.7). There was no significant difference in seroconversion rates between the adjuvanted and non-adjuvanted NmCV-5 groups for any serogroup (table 3).

The proportions of participants with baseline titres of at least eight and at least 128 against serogroups C, Y, and W were low (≤ 12 [$\leq 60\%$]); there were high proportions of individuals with baseline titres at those thresholds or more against serogroup A in the non-adjuvanted and adjuvanted NmCV-5 vaccine groups (15 [75%] and 17 [85%], respectively) compared with control (ten [50%]); the lowest proportions of baseline titres at those thresholds were against serogroup X (≤ 5 [$\leq 25\%$]). After vaccination, 100% (20/20) of the non-adjuvanted NmCV-5 group had titres of eight or more and of 128 or more for all five serogroups; 19 (95%) to 20 (100%) participants in

	Total (n=60)	Adjuvanted NmCV-5* (n=20)	Non-adjuvanted NmCV-5 (n=20)	Menactra† (control; n=20)
Local symptoms				
Pain	29 (48%)	12 (60%)	10 (50%)	7 (35%)
Mild	..	10 (50%)	6 (30%)	7 (35%)
Moderate	..	2 (10%)	4 (20%)	0
Erythema	2 (3%)	0	1 (5%)	1 (5%)
Mild	..	0	1 (5%)	0
Moderate	..	0	0	1 (5%)
Induration	3 (5%)	1 (5%)	1 (5%)	1 (5%)
Mild	..	1 (5%)	1 (5%)	1 (5%)
Moderate	..	0	0	0
Systemic symptoms				
Fever	0	0	0	0
Headache	11 (18%)	5 (25%)	3 (15%)	3 (15%)
Mild	..	2 (10%)	2 (10%)	3 (15%)
Moderate	..	3 (15%)	1 (5%)	0
Malaise	8 (13%)	3 (15%)	3 (15%)	2 (10%)
Mild	..	1 (5%)	2 (10%)	2 (10%)
Moderate	..	2 (10%)	1 (5%)	0
Arthralgia	4 (7%)	1 (5%)	1 (5%)	2 (10%)
Mild	..	1 (5%)	0	2 (10%)
Moderate	..	0	1 (5%)	0
Myalgia	11 (18%)	2 (10%)	6 (30%)	3 (15%)
Mild	..	1 (5%)	4 (20%)	2 (10%)
Moderate	..	1 (5%)	2 (10%)	1 (5%)
Diarrhoea	6 (10%)	3 (15%)	1 (5%)	2 (10%)
Mild	..	3 (15%)	1 (5%)	2 (10%)
Moderate	..	0	0	0
Anorexia	2 (3%)	1 (5%)	0	1 (5%)
Mild	..	1 (5%)	0	1 (5%)
Moderate	..	0	0	0
Chills	3 (5%)	1 (5%)	1 (5%)	1 (5%)
Mild	..	1 (5%)	0	1 (5%)
Moderate	..	0	1 (5%)	0
Vomiting	2 (3%)	0	1 (5%)	1 (5%)
Mild	..	0	1 (5%)	1 (5%)
Moderate	..	0	0	0

*Aluminium-phosphate adjuvanted NmCV-5 vaccine. †Quadrivalent meningococcal conjugate vaccine Menactra (MCV4).

Table 2: Maximum reactogenicity within 7 days of vaccination

the adjuvanted NmCV-5 group achieved a response. As expected, no additional seroprotective responses were elicited against serogroup X in the control Menactra group; however, 18 (90%) to 20 (100%) participants in this group achieved an rSBA titre of eight or more after vaccination against serogroups A, C, Y, and W (table 3). Apart from the NmCV-5 groups versus control, there were no significant differences in the proportions of participants with titres of eight or more, or 128 or more.

At baseline, the GMT against serogroup A for the non-adjuvanted NmCV-5 group, but not the adjuvanted NmCV-5 group, was significantly higher than that for the control group; 95% CIs for the difference in GMTs against

	Serogroup A (n=20)	Serogroup C (n=20)	Serogroup Y (n=20)	Serogroup W (n=20)	Serogroup X (n=20)
Post-vaccination seroconversion*					
Adjuvanted NmCV-5†	17 (85%), 62.1-96.8	19 (95%), 75.1-99.9	19 (95%), 75.1-99.9	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9
Non-adjuvanted NmCV-5	14 (70%), 45.7-88.1	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9	19 (95%), 75.1-99.9	17 (85%), 62.1-96.8
Menactra‡ (control)	16 (80%), 56.3-94.3	17 (85%), 62.1-96.8	15 (75%), 50.9-91.3	16 (80%), 56.3-94.3	1 (5%), 0.1-24.9
Sero-protection, 1/8§					
Baseline					
Adjuvanted NmCV-5†	15 (75%), 50.9-91.3	9 (45%), 23.1-68.5	6 (30%), 11.9-54.3	5 (25%), 8.7-49.1	5 (25%), 8.7-49.1
Non-adjuvanted NmCV-5	17 (85%), 62.1-96.8	3 (15%), 3.2-37.9	9 (45%), 23.1-68.5	9 (45%), 23.1-68.5	3 (15%), 3.2-37.9
Menactra‡ (control)	10 (50%), 27.2-72.8	4 (20%), 5.7-43.7	12 (60%), 36.1-80.9	8 (40%), 19.1-63.9	2 (10%), 1.2-31.7
28 days post-vaccination					
Adjuvanted NmCV-5†	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9
Non-adjuvanted NmCV-5	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0
Menactra‡ (control)	20 (100%), 83.2-100.0	18 (90%), 68.3-98.8	20 (100%), 83.2-100.0	18 (90%), 68.3-98.8	2 (10%), 1.2-31.7
Sero-protection, 1/128¶					
Baseline					
Adjuvanted NmCV-5†	15 (75%), 50.9-91.3	5 (25%), 8.7-49.1	6 (30%), 11.9-54.3	5 (25%), 8.7-49.1	3 (15%), 3.2-37.9
Non-adjuvanted NmCV-5	17 (85%), 62.1-96.8	2 (10%), 1.2-31.7	9 (45%), 23.1-68.5	9 (45%), 23.1-68.5	3 (15%), 3.2-37.9
Menactra‡ (control)	10 (50%), 27.2-72.8	4 (20%), 5.7-43.7	12 (60%), 36.1-80.9	7 (35%), 15.4-59.2	2 (10%), 1.2-31.7
28 days post-vaccination					
Adjuvanted NmCV-5†	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9	19 (95%), 75.1-99.9	20 (100%), 83.2-100.0	19 (95%), 75.1-99.9
Non-adjuvanted NmCV-5	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0	20 (100%), 83.2-100.0
Menactra‡ (control)	20 (100%), 83.2-100.0	17 (85%), 62.1-96.8	20 (100%), 83.2-100.0	18 (90%), 68.3-98.8	2 (10%), 1.2-31.7
GMT (95% CI)					
Baseline					
Adjuvanted NmCV-5†	187.0 (49.6-708.0)	9.85 (3.77-25.70)	10.20 (3.03-34.40)	8.00 (2.44-26.20)	6.28 (2.44-16.10)
Non-adjuvanted NmCV-5	350 (119-1025)	4.29 (2.01-9.15)	24.3 (6.16-95.4)	26.90 (6.19-117.00)	5.28 (1.89-14.80)
Menactra‡ (control)	33.10 (8.42-130.00)	5.66 (2.15-14.90)	53.8 (14.2-204.0)	13.90 (4.16-46.60)	3.36 (1.76-6.43)
28 days post-vaccination					
Adjuvanted NmCV-5†	6889 (3767-12 596)	4096 (1720-9756)	4545 (1700-12 149)	8192 (3439-19 513)	1351 (577-3165)
Non-adjuvanted NmCV-5	5595 (3324-9418)	6208 (3579-10 771)	9410 (4935-17 942)	11 191 (6720-18 635)	1607 (892-2895)
Menactra‡ (control)	3214 (1978-5222)	388 (139-1085)	2353 (1302-4251)	1261 (388-4091)	3.14 (1.73-5.71)

Data are n (%) 95% CI, or geometric means (95% CI). *Seroconversion defined as an increase of four times or greater in rabbit complement-dependent serum bactericidal activity (rSBA) titre post-vaccination. †Aluminium-phosphate adjuvanted NmCV-5 vaccine. ‡Quadrivalent meningococcal conjugate vaccine Menactra (MCV4). §Sero-protection defined as the proportion with an rSBA titre of ≥8. ¶Sero-protection defined as the proportion with an rSBA titre of ≥128. ||Geometric mean titre (GMT) of rSBA.

Table 3: Meningococcal vaccine immunogenicity as measured by rabbit complement serum bactericidal activity

control were 0.89–35.8 (not significant) for the adjuvanted vaccine and 1.96–56.9 (significant) for non-adjuvanted vaccine. Baseline GMTs for the other serogroups were similar among the three vaccine groups. After vaccination, GMTs were significantly greater in the two NmCV-5 groups versus control for all serogroups, except for adjuvanted NmCV-5 versus control for serogroup A and non-adjuvanted NmCV-5 versus control for serogroup Y (table 3). Differences between the two NmCV-5 groups were not significant. However, because of the low baseline titres in the control group against serogroup A, when the geometric mean-fold rise (GMFR) from baseline was calculated, the serogroup A response in the control group was higher than in the two NmCV-5 groups (appendix). Thus, a lower baseline titre against serogroup A was more likely to give a response that was several times higher. The baseline GMT was 33.1 for control, 187.0 for adjuvanted NmCV-5, and 350.0 for non-adjuvanted NmCV-5; day 29

GMTs were 3214 for control, 6889 for adjuvanted NmCV-5, and 5595 for non-adjuvanted NmCV-5. GMTs and GMFR for all remaining serogroups were higher in the NmCV-5 groups than in the control group. Only the non-adjuvanted NmCV-5 group achieved 100% sero-protection (ie, all 20 participants achieved titres ≥128 times) against all five serogroups.

Discussion

This first-in-human study showed that two different formulations of the candidate pentavalent meningococcal conjugate vaccine NmCV-5 were not associated with substantial local or systemic reactogenicity or other safety signals that might preclude its continued development. At 4 weeks following vaccination, all three study vaccines (adjuvanted NmCV-5, non-adjuvanted NmCV-5, and Menactra control) elicited antibody responses to *N meningitidis* serogroups A, C, Y, and W. As designed,

the adjuvanted and non-adjuvanted NmCV-5 vaccines were also able to elicit robust antibody responses to serogroup X. The four-times or greater rises in rSBA titres in response to vaccination for all five serogroups were similar for the two NmCV-5 groups.

A limitation of the study was the small sample size; as such, the data did not provide conclusive information on the comparative immunogenicity of the two NmCV-5 formulations. There was no evidence to suggest that either adjuvanted or non-adjuvanted NmCV-5 is likely to be inferior to Menactra, and also no evidence to suggest that non-adjuvanted NmCV-5 is likely to be inferior to adjuvanted NmCV-5, but our study was not powered to assess this. To assess the potential protective immune responses of these two formulations of NmCV-5 in toddlers (aged 12–16 months), we started a phase 2 study in Mali (ClinicalTrials.gov Identifier NCT03295318).

A large proportion of participants had baseline rSBA titres of 128 or more for the different serogroups; these baseline proportions were highest for serogroup A for participants in all three vaccine groups. Although a history of meningococcal vaccination or previous meningococcal infection were exclusion criteria for the study, the high baseline titres might have been attributable to previous subclinical infection or erroneous histories provided by study participants. The high baseline rSBA titres against serogroup A could also have been caused by antibodies elicited by antigenically cross-reacting polysaccharides on *Haemophilus influenzae* type b and some strains of the genus *Bacillus*, which could compose parts of normal flora.³³ High baseline rSBA titres have occurred in trials of other meningococcal conjugate vaccines.^{34–36}

The human complement-dependent SBA (hSBA) assay was originally recognised as the serological correlate of protection for *N meningitidis* serogroup C.²⁴ However, the practical and technical complexity of obtaining and standardising hSBA for these assays led to the modified approach of using baby rabbit complement for SBA assays. On one hand, hSBA assays might have reduced sensitivity for *N meningitidis* serogroup C³⁷ and although titres of four or more with this assay are predictive of protection, titres of less than four might not necessarily predict susceptibility to serogroup C.^{38–40} The correlation between hSBA and rSBA is strong for serogroup C but weak for serogroup A.⁴¹ On the other hand, three monovalent serogroup C vaccines were licensed in Europe in 1999 and 2000 based on rSBA responses, and during post-licensure surveillance, rSBA titres of eight or more were confirmed as the antibody threshold that best correlated to vaccine effectiveness.³⁰ Furthermore, the licensure of MenAfriVac was completed using the rSBA assay. Therefore, there is reasonable justification for the use of the rSBA assay as an immunological outcome measure in our study. The only correlate of protection is an rSBA titre of eight or more for serogroup C; there is no established correlate of protection for serogroups A,

W, Y, or X. However, significant rSBA titres against serogroup X have been observed during outbreaks in 2007.²⁷

The striking reduction of serogroup A invasive disease since the introduction of MenAfriVac has generated hope for the potential for elimination of meningococcal disease outbreaks in sub-Saharan Africa. By contrast with the annual incidence of meningococcal disease of more than 100 cases per 100 000 individuals before 2010, there were only two cases of invasive serogroup A disease documented for 2017.⁷ Therefore, there is enthusiasm for a multivalent meningococcal conjugate vaccine to further reduce the incidence of invasive meningococcal disease in sub-Saharan Africa. Despite the global availability of quadrivalent meningococcal conjugate vaccines, the high cost per dose of these vaccines has made their routine use in sub-Saharan Africa implausible. The availability of a multivalent meningococcal vaccine that also includes serogroup X is likely to be relevant for developed countries, especially for travellers to sub-Saharan Africa and during the Hajj. Using the same roadmap paved by the development of the MenAfriVac, an affordable pentavalent meningococcal vaccine has the potential to eliminate epidemic meningococcal disease from sub-Saharan Africa.

Contributors

WHC, KMN, LM, NH, FML, AC, PSK, VB, MRA, and MLM participated in the study design. WHC, KMN, CRB, MFP, and MKR participated in data collection. RB and HF were responsible for laboratory testing. WHC, CRB, VB, and MLM, participated in data cleaning. WHC, KMN, MFP, NH, FML, RMD, SSP, AC, PSK, RB, LD, and MRA participated in data analysis and interpretation. WHC, KMN, MFP, NH, AC, PSK, RB, HF, LD, and MRA participated in the primary manuscript writing. All authors contributed to the final published manuscript.

Declaration of interests

FML, AC, RMD, SSP and PSK are employed by the Serum Institute of India Pvt, Pune. All other authors declare no competing interests.

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