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Protocol for a phase IV double-blind randomised controlled trial to investigate the effect of the 13-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine on pneumococcal colonisation using the experimental human pneumococcal challenge model in healthy adults (PREVENTING PNEUMO 2)

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ABSTRACT

Introduction Despite widely available vaccinations, *Streptococcus pneumoniae* (SPN) remains a major cause of morbidity and mortality worldwide, causing community-acquired pneumonia, meningitis, otitis media, sinusitis and bacteraemia. Here, we summarise an ethically approved protocol for a double-blind, randomised controlled trial investigating the effect of the 13-valent pneumococcal conjugate vaccine (PCV13) and the 23-valent pneumococcal polysaccharide vaccine (PPV23) on pneumococcal nasopharyngeal colonisation acquisition, density and duration using experimental human pneumococcal challenge (EHPC).

Methods and analysis Healthy adult participants aged 18–50 years will be randomised to receive PCV13, PPV23 or placebo and then undergo one or two EHPCs involving intranasal administration of SPN at 1-month post-vaccination with serotype 3 (SPN3) and 6 months with serotype 6B (SPN6B). Participants randomised to PCV13 and placebo will also be randomised to one of two clinically relevant SPN3 strains from distinct lineages within clonal complex 180, clades Ia and II, creating five study groups. Following inoculation, participants will be seen on days 2, 7, 14 and 23. During the follow-up period, we will monitor safety, colonisation status, density and duration, immune responses and antigenuria. The primary outcome of the study is comparing the rate of SPN3 acquisition between the vaccinated (PCV13 or PPV23) and unvaccinated (placebo) groups as defined by classical culture. Density and duration of colonisation, comparison of acquisition rates using molecular methods and evaluation of the above measurements for individual SPN3 clades and SPN6B form the secondary objectives. Furthermore, we will explore the immune responses

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The experimental human pneumococcal challenge (EHPC) model is a platform for evaluating vaccine efficacy against pneumococcal colonisation—a prerequisite for development of pneumococcal disease—and requires significantly smaller numbers of participants than a field trial.
⇒ This is the first time a study will compare the two most commonly used pneumococcal vaccines globally against placebo, using the same outcome measures, and aims to identify associated immunological parameters that potentially correlate with protection against pneumococcal colonisation.
⇒ Our study will explore why *Streptococcus pneumoniae* serotype 3 (SPN3) remains a leading cause of pneumococcal disease globally despite its inclusion in both the 13-valent pneumococcal conjugate vaccine (PCV13) and 23-valent pneumococcal polysaccharide vaccine (PPV23) and PCV13's documented efficacy against SPN3 invasive pneumococcal disease and non-bacteraemic pneumonia.
⇒ As a decrease in colonisation density potentially translates to a decreased risk of disease and community transmission, we will be able to explore direct and indirect vaccine protection by measuring density against the defined EHPC timeline.
⇒ This is an experimental model using one serotype at each challenge, at a fixed inoculum dose, which only approximates natural colonisation and therefore may not be fully generalisable.

associated with these vaccines, their effect on colonisation and the relationship between colonisation and urinary pneumococcal antigen detection.

Ethics and dissemination The study is approved by the NHS Research and Ethics Committee (Reference: 20/NW/0097) and by the Medicines and Healthcare products Regulatory Agency (Reference: CTA 25753/0001/001–0001). Findings will be published in peer-reviewed journals.

Trial registration number ISRCTN15728847, NCT04974294.

INTRODUCTION

Colonisation of the human upper respiratory tract by *Streptococcus pneumoniae* (SPN) is frequent, with a prevalence of 40%–95% among infants,[1] 10%–25% among adults aged ≥18 years[1–4] and 0%–10% among adults aged ≥65 years.[4 5] Pneumococci adhere to the epithelial cells of the nasopharynx, and in most cases, this does not lead to symptomatic pneumococcal disease.[6 7] However, pneumococcal nasopharyngeal colonisation is considered a prerequisite for pneumococcal disease. Certain groups, such as children ≤5 years, the immunosuppressed or older adults, are at higher risk of developing symptomatic pneumococcal disease following colonisation.

Controlled human infection models & Experimental human pneumococcal challenge model

Controlled human infection models (CHIMs) have been vital in vaccine development and exploring host-pathogen interactions, a notable example being the licensure of the first malaria vaccine.[8] CHIMs typically recruit young, healthy volunteers with a low risk of severe outcomes. Our experimental human pneumococcal challenge (EHPC) model allows testing vaccine efficacy (VE) against experimental colonisation in a timely and cost-effective manner, requiring fewer participants than phase III clinical trials.[9–11] It enables the assessment of immunological responses to nasopharyngeal colonisation and vaccine-induced correlates of protection against SPN. Participants are given predefined SPN doses by nasal administration, with some becoming colonised at a duration and density typical of natural colonisation episodes. Samples including nasal washes (NW), nasal cells and blood are taken to assess colonisation and anti-pneumococcal immune responses.

Over 1400 participants have been challenged with several strains in different age cohorts and at various doses (10 000–320 000 colony forming units (CFU)/100 µL). Selected strains are fully sequenced and antibiotic sensitive. Careful participant selection and exclusion criteria reduce the risk of pneumococcal disease.

Pneumococcal vaccines

Two vaccines are mainly recommended to prevent pneumococcal infections: 23-valent pneumococcal polysaccharide vaccine (PPV23) (Pneumovax, MSD) and 13-valent pneumococcal conjugate vaccine (PCV13) (Prevenar, Pfizer). PPV23 contains capsular polysaccharides from 23 serotypes.[12] PCV13 contains purified polysaccharides of capsular antigens of 13 serotypes individually conjugated with a genetically detoxified diphtheria toxin referred to as cross-reactive material (CRM197).[13] Both include serotype 3. The WHO recommends including PCVs in all childhood national immunisation programmes (NIP).[14] High-income and middle-income countries use PPV23 and PCV13 for the elderly and at-risk and immunosuppressed adult groups,[15] with countries varying on using PPV23 alone, PCV13 alone or in combination.[16 17] Countries are also starting to recommend newer PCVs for use in adults.[18]

Systematic reviews and meta-analyses support the efficacy of PPV23 against invasive pneumococcal disease (IPD); however, VE against non-bacteraemic pneumococcal pneumonia is uncertain.[19–23] The current consensus is that PPV23 does not offer protection against natural colonisation.[19] Effects in CHIMs have yet to be established.

Randomised controlled trial (RCT) data support PCV effectiveness against IPD,[24] nasopharyngeal colonisation in children and adults[25 26] and vaccine-type (VT) non-bacteraemic pneumonia in older adults.[24] Other analyses also indicate protection against all-cause pneumonia and lower respiratory tract infection,[27–29] and otitis media in children.[30]

Epidemiology, SPN3 and PCV impact

Regional UK data showed an IPD incidence increase between 2014 and 2018, primarily due to non-PCV13 VTs in all age groups, especially in adults and older adults. SPN 8, 12F and 9N (contained in PPV23, not PCV13) were responsible for 37.5% of IPD cases. However, PCV13 VTs were responsible for 20.1%, mainly due to SPN serotype 3 (SPN3) (45.9% of PCV13-type disease).[31]

For individuals, evidence indicates PCV13 protects against SPN3. A post hoc analysis of RCT data showed a 61.5% (95% CI: 17.6% to 83.4%) reduction in clinical community-acquired pneumonia due to SPN3 in older adults,[32] while a meta-analysis showed protection against SPN3 IPD in children.[33] The sole RCT of PCV13 and colonisation in children found no reduction in SPN3 colonisation,[25] but CIs were wide, without data on density. These data are essential, as decreasing density might be the primary mechanism of reducing disease and transmission.[34]

Interpreting the population-level impact of PCV13 against SPN3 disease is challenging. The impact depends on direct and indirect vaccine effects and other factors such as vaccination rates, the activity of co-circulating respiratory viruses, changes in antibiotic resistance rates associated with serotypes or the prevalence of immunocompromising conditions. From 2000 to 2017 in the UK, SPN3 IPD cases remained stable in children aged ≤5 years after introducing PCV7 (not containing SPN3) and subsequently PCV13 (containing SPN3) in the NIP.[35] Contrarily, in the whole UK population, SPN3 increased modestly following PCV7 introduction in the childhood NIP, declined following the introduction of PCV13 and

then after several years increased to above pre-PCV7 levels.[36] Furthermore, data from PCV10-using countries (not containing SPN3) versus PCV13-using countries in NIPs suggests small reductions in SPN3 IPD among unvaccinated cohorts in PCV13-using countries compared with an increase in PCV10-using countries, suggesting some impact on transmission.[36]

Lower population-level protection may result from several hypothetical mechanisms. Limited vaccine impact on SPN3 colonisation is possible. This limitation could result from specific characteristics of the SPN3 capsule: SPN3 grows as a mucoid colony and sheds its non-covalently bound capsule, seemingly as a defence against antibody-mediated phagocytosis,[37 38] which, in theory, can overwhelm the protective capacity of vaccine-induced antibodies. Post-vaccination antibody levels are lower for SPN3 than other PCV13 serotypes and may not substantially reduce colonisation.[25]

Clades Ia and II

Pneumococci can be classified into phylogenetic lineages through analysis of the nucleotide diversity in the core genome. Lineages include strains with the same multilocus sequence typing (MLST) and serotype. MLSTs can be further grouped in clonal complexes (CC). Most SPN3 isolates globally belong to a single CC (CC180), containing multiple lineages. One of them is clade Ia, which historically has accounted for most genomes studied (primarily European isolates).[39] Azarian *et al* reported that before introducing PCV13 into paediatric NIPs globally, most SPN3 isolates fell into clade Ia.[39] Almost simultaneously with global PCV adoption into paediatric NIPs, clade II lineage was identified in Asia and spread globally. However, the study showed no clear association with PCV use at country level or the timing of PCV13 introduction and the emergence of clade II. Clade II is now the dominant type in some locations: a UK study demonstrated that following childhood PCV13 introduction in 2010, SPN3 clade Ia declined in all age groups.[40] A significant increase in SPN3 clade II occurred some years later, even while clade Ia remained at lower levels than pre-PCV13. Clade II has a higher prevalence of genes associated with antibiotic resistance, possibly contributing to its fitness and spreading success.[39] Overall, the mechanisms for the rise of clade II remain uncertain.

Longer-term protection of PCV13 against colonisation

The PCV13 EHPC study showed short-term protection against colonisation at 4 weeks post-vaccination.[41] However, almost no data exist on whether PCV13 confers longer-term protection against colonisation in healthy adults over an extended period. An RCT study in the Netherlands, among adults ≥65 years, reported that PCV13 elicited a temporary reduction in VT-pneumococcal colonisation at 6 months post-vaccine but no significant protection at 12 and 24 months.[42] However, density and duration were not measured at any time point. We are not expecting any heterologous protection against SPN serotype 6B (SPN6B) acquisition following the SPN3 challenge in part A. Data have shown protection following a homologous rechallenge,[43 44] but not heterologous rechallenge of naturally colonised volunteers,[45] or experimental challenge with two different strains (Connor *et al*, unpublished data).

Rationale

Using the EHPC model, we will investigate gaps in knowledge around the immunity induced by PCV13 and PPV23 vaccination, with a focus on short-term direct protection against SPN3 colonisation and longer-term protection against SPN6B colonisation, using the endpoints of both colonisation acquisition as a dichotomous variable and colonisation density and duration as continuous variables. Given the available epidemiological data reporting SPN3 clade Ia and clade II IPD incidence pre-PCV13 and post-PCV13 implementation, we anticipate PCV13's protection against clade Ia to be greater than against clade II.

Furthermore, we will undertake a detailed characterisation of immune responses for both vaccines to identify potential immunological correlates for PCV's impact on colonisation and assess the longer-term impact of pneumococcal vaccines on colonisation.

Study objectives

The primary objective is to compare the SPN3 acquisition rate at 1 month post pneumococcal vaccination to placebo vaccination during the 23-day follow-up period following EHPC. Secondary objectives include the same comparison for SPN6B at 6 months post-vaccination, comparing the acquisition rate of clades of SPN3 colonisation (two clades for PCV13 and Ia only for PPV23) and the density and duration of SPN3 and SPN6B colonisation following inoculation. Exploratory objectives include comparing immune responses between the two pneumococcal vaccines, characterising the immune changes as a response to vaccination and colonisation, assessing pneumococcal urine antigen detection (UAD) levels, and continuous safety assessment of the EHPC model. For objectives and outcome measures, see table 1.

METHODS AND ANALYSIS

Study overview

This is a double-blind RCT in which participants will be randomised to either receive PCV13, PPV23 or placebo (saline) and undergo two EHPCs with intranasal SPN administration at 1 month (part A) and 6 months (part B).

SPN3 strains will be used for EHPC in part A (strains PFESP306 (clade Ia) and PFESP505 (clade II)). Both SPN3 strains were isolated from patients with IPD. PFESP306 was isolated from a pleural fluid sample in the USA, and PFESP505 from blood cultures in a patient in Asia. Both were used in our recently completed dose-characterisation study (Establishing SPN3 Challenge Model, Research and

Table 1 Objectives and outcome measures

	Objectives	Outcome measures
Primary	To compare the rate of acquisition of experimental *Streptococcus pneumoniae* (SPN) serotype 3 (SPN3) colonisation for the 23 days following experimental human pneumococcal challenge (EHPC) in persons 1 month post pneumococcal vaccination (PCV-13 or PPV-23) to controls defined by classical culture from nasal wash (NW).	The rate of experimental SPN3 colonisation determined by the presence of experimental SPN3 clades (combined) in NW by classical culture at ANY time point (D2, 7, 14, 23) following EHPC 1 month after vaccination in PCV-13 vs control and PPV-23 vs control.
Secondary	To determine the density and duration of experimental SPN3 colonisation at EACH and ANY time point for SPN3 clades (combined and individually) for 23 days following EHPC at 1 months post pneumococcal vaccination (PCV-13 or PPV-23) by classical culture and molecular methods from NW.	The density of experimental SPN3 colonisation (both and each clade) in NW at each and any time point (D2, 7, 14, 23) by classical culture and PCR (combined and individually) following EHPC at 1 months after vaccination in PCV-13 vs control, and PPV-23 vs control.
		The duration of experimental pneumococcal colonisation with two clades of SPN3 at 1 month post vaccination determined by the last NW following EHPC in which pneumococcus is detected, by classical culture and by molecular methods (combined and individually) in the PCV-13, PPV-23 and control groups.
	To compare the rate of acquisition of experimental SPN3 colonisation at EACH time point for SPN3 clades (combined and individually— two clades for PCV-13, one for PPV-23) for 23 days following EHPC in persons 1 month post pneumococcal vaccination (PCV-13 or PPV-23) to controls defined by classical culture and by molecular methods from NW.	The rate of experimental SPN3 colonisation determined by the presence of experimental SPN3 clades (combined and individually) in NW by classical culture and PCR (combined and individually) at EACH time point (D2, 7, 14, 23) following EHPC 1 month after vaccination in PCV-13 vs control, and PPV-23 vs control.
	To compare the rate of acquisition of experimental SPN3 colonisation at ANY time point for EACH SPN3 clade individually for 23 days following EHPC in persons 1 month post pneumococcal vaccination (PCV-13 or PPV-23) to controls defined by classical culture and by molecular methods from NW.	The rate of experimental SPN3 colonisation determined by the presence of EACH experimental SPN3 individually in NW by classical culture and PCR (combined and individually) at ANY time point (D2, 7, 14, 23) following EHPC 1 month after vaccination in PCV-13 vs control, and PPV-23 vs control.
	To compare the rate of acquisition of experimental SPN3 colonisation at ANY time point for BOTH SPN3 clades combined following EHPC in persons 1 month post pneumococcal vaccination (PCV-13 or PPV-23) to controls defined by molecular methods from NW.	The rate of experimental SPN3 colonisation determined by the presence of experimental SPN3 clades (combined and individually) in NW by PCR at ANY time point (D2, 7, 14, 23) following EHPC 1 month after vaccination in PCV-13 vs control, and PPV-23 vs control.
	To compare the rate of acquisition of experimental SPN6B colonisation at EACH and ANY time point during the 23 days following EHPC in persons 6 months post pneumococcal vaccination (PCV-13 or PPV-23) to controls defined by classical culture and molecular methods from NW.	The rate of experimental SPN6B colonisation determined by the presence of experimental SPN6B in NW by classical culture and PCR (combined and individually) at EACH and ANY time point (D2, 7, 14, 23) following EHPC 6 months after vaccination in PCV-13 vs control and PPV-23 vs control.
	To determine the density and duration of SPN6B experimental colonisation (PCV-13 or PPV-23) EACH and ANY time point for 23 days following EHPC at 6 months post pneumococcal vaccination (PCV-13 or PPV-23) by classical culture and molecular methods from NW.	The density of experimental SPN6B colonisation in NW at each and any time point (D2, 7, 14, 23) by classical culture and PCR (combined and individually) following EHPC at 6 months after vaccination in PCV-13 vs control, and PPV-23 vs control.
		The duration of experimental pneumococcal colonisation with SPN6B at 6 months post vaccination determined by the last NW following EHPC in which pneumococcus is detected, by classical culture and by molecular methods (combined and individually) in the PCV-13, PPV-23 and control groups.
Exploratory	To compare immune responses of those who receive PCV-13 vs PPV-23. The immune responses to be assessed include but are not limited to: levels of polysaccharide-specific SPN3 and SPN6B memory B cells, levels and function of antibody responses (OPA and agglutination capacity) pre-vaccination and post-vaccination (pre-challenge) at 1 and 6-month in serum, PBMCs and NW.	Measurement of anti-pneumococcal polysaccharide specific IgA levels in serum and NW samples using ELISAs.
		Quantification of antibody-mediated agglutination in NW samples at the above time points using a flow-cytometry based antibody agglutination assays.
		Measurement of opsonophagocytic activity (OPA) of antibodies in sera before and after vaccination.
		Determination of the number of SPN3-polysaccharide and SPN6B-polysaccharide specific memory B cells in PBMC samples using ELISpots. This will be complimented by multicolour flow cytometry to quantify these cells and characterise the B cell populations with regard to their maturity and activation.
		Other relevant immunological mechanisms may also be evaluated using appropriate samples and processing techniques.
	To characterise immune cell populations and dynamics in response to vaccination and experimental inoculation in nasal cell samples.	Investigation of the changes in nasal cell populations using immunophenotyping methods including multicolour flow cytometry to identify cells such as neutrophils, monocytes, T cells and B cells in nasal mucosa at screen and re-screen, Day 2 and 7 following both inoculations.
	To characterise the transcriptional changes in immune cells in response to vaccination and experimental inoculation.	Determination of gene induction and regulation to identify patterns in vaccine responders and non-responders, and in individuals who become experimentally colonised vs those who remain protected.
	To determine if persons with colonisation (defined as experimental or natural SPN acquisition at any time point) have different antigen levels (UAD test) from those without colonisation to help inform interpretation of UAD for pneumonia.	Comparison of cut points of the UAD test for SPN colonisation (natural or experimental or combined) from urine samples collected at re-screen, Day 2 and Day 7 for both inoculations.
	To correlate pneumococcal colonisation in healthy participants with pneumococcal antigen detection in urine pre-inoculation and post-inoculation.	Correlation of SPN nasal colonisation (natural or experimental or combined) with of SPN antigen detection in urine samples collected at re-screen, Day 2 and Day 7 for both inoculations.
	To describe symptoms following EHPC with SPN3 (both clades) and SPN6B.	The presence of mild or moderate symptoms as recorded on a Likert scale in participants with SPN3 and SPN6B within the first 7 days after EHPC. Sore throat grading score will also be used if applicable.
	To determine if PCV-13 or PPV-23 lead to a reduction in antibiotic use in the follow-up period for participant symptoms potentially related to the EHPC with SPN3 and SPN6B (by the clinical study team using a pre-defined assessment algorithm).	Comparison of rate of antibiotic courses during the 23 days following EHPC with SPN3 and SPN6B between the PCV-13, PPV-23 and placebo arms (excluding those who withdraw early or have early antibiotic treatment for non-clinical reasons like travel).
	To compare the requirement for antibiotics initiated in the follow-up period for participant symptoms potentially related to the EHPC (by the clinical study team using a pre-defined assessment algorithm) between the SPN3 and SPN6B groups in unvaccinated persons (saline control group).	Comparison of rate of antibiotic courses prescribed due to symptoms potentially related to EHPC at any time point during the 23 days following challenge with SPN3 at 1 month post vaccination and with SPN6B at 6 months post vaccination in the control group (excluding those who withdraw early or have early antibiotic treatment for non-clinical reasons like travel).

PBMC, peripheral blood mononuclear cells; PCV13, 13-valent pneumococcal conjugate vaccine ; PPV23, 23-valent pneumococcal polysaccharide vaccine; SPN6B, SPN serotype 6B; UAD, urine antigen detection .

Ethics Committee (REC) reference 19/NW/0238, unpublished data). In that study, 96 participants were inoculated with doses ranging from 10 000 to 160 000 CFUs to establish an optimal dose. In PREVENTING PNEUMO 2, an inoculum dose of 80 000 CFUs will be used for both strains, expecting a 30%–40% colonisation rate for SPN3. When establishing the SPN3 challenge model, a proportion of participants developed mild symptoms, mostly pharyngitis, particularly at higher doses of SPN3 inoculum (160 000 CFUs). All participant's symptoms resolve following 3–5 days of oral amoxicillin with no long-term sequelae. In PREVENTING PNEUMO 2, we will use the inoculation dose of 80 000 CFUs that had lower colonisation rates (30% for clade Ia and 40% for clade II) than the higher dose of 160 000 CFUs (40% for clade Ia and 70% for clade II) but caused significantly fewer symptoms.

SPN6B will be used for the EHPC in part B (strain BHN418, GenBank accession number ASHP00000000.1). This strain was isolated from a child with otitis media in the Netherlands. We have used this strain in our previous studies, and we predict a 40% colonisation rate at an inoculum dose of 80 000 CFUs.[41 46 47]

All strains are sensitive to amoxicillin, verified with laboratory testing by Public Health England (PHE).

After each inoculation, participants will be followed-up for 23 days, on days 2, 7, 14 and 23. Colonisation presence, density and duration and immune responses will be assessed during the follow-up period. We will continuously evaluate the safety of the model.

The study is expected to run from July 2021 to January 2023.

Study participants

Recruitment

Healthy volunteers aged 18–50 will be recruited using various methods, such as but not limited to: social media, volunteer databases, university campuses and halls, newsletters via employers and General Practitioner (GP) surgeries. Participants will be remunerated for their time, travel and inconvenience.

Study setting

Study visits and procedures will occur at the Accelerator Research Building, Liverpool. If a participant develops symptoms consistent with Coronavirus Disease 2019 (COVID-19), they will be assessed in a designated area in the Liverpool University Hospitals Foundation Trust. The study will follow local safety protocols and standard operating procedures regarding COVID-19.

Eligibility

We will exclude participants with health conditions that would increase their risk of infection following inoculation. We will also exclude participants in regular close contact with children under the age of 5, immunosuppressed individuals, or those deemed extremely vulnerable by PHE as a response to the COVID-19 pandemic. Participants allergic to penicillin or amoxicillin and those who have previously received a pneumococcal vaccine will also be excluded. Exclusion and inclusion criteria can be found in tables 2 and 3.

Participant retention

Close personal contact with participants will be maintained throughout, engaging and sustaining interest. We use an online booking system to ensure reminders are sent via various routes. Remuneration is paid at the ends of parts A and B and is weighted more towards Part B to encourage completion of both study parts.

Study timeline

Participation will begin from the day of screening/enrolment (up to 12 days before vaccination) and continue until day 51 after vaccination (subjects participating in part A only) or day 191 after vaccination (subjects participating in part A and B). The maximum duration of participation is approximately 7 months. The study timeline is summarised in figure 1. More information about the visits and sampling schedule is depicted in table 4.

Part A

Initial visit

Potential participants will attend an initial visit at the site or virtually. At this visit, all study procedures will be explained. They will get the opportunity to ask questions either in a group or one-to-one and give informed consent (see online supplemental appendix 1) to a registered healthcare professional (doctor or nurse) after completing a questionnaire to check their understanding. A medical history will be taken to determine their eligibility. Screening blood tests will be collected during this or the screening visit.

These visits might occur simultaneously or sequentially with a maximum interval of 12 days. During screening, eligibility will be re-confirmed by a medical doctor after reviewing the initial visit electronic case report form, vital signs, pregnancy test and screening blood tests if applicable, and performing a clinical examination. Sampling will be carried out to establish a microbiological and immunological baseline. NW samples will be collected to assess natural pneumococcal colonisation, and sera, peripheral blood mononuclear cells (PBMCs) and nasal cells will be collected (with mucosal curette) to establish an immunological baseline. Nasal cells and blood will also be used for a baseline transcriptomic analysis. During the vaccination visit, participants will be randomised to either PCV13, PPV23 or placebo and the SPN3 inoculum (clades Ia and II for PCV13 and placebo, clade Ia only for PPV23).

Assignment of interventions and blinding

An independent Liverpool School of Tropical Medicine (LSTM) coordinator will generate the randomisation schedule. Two staff members not involved in the study's conduct will produce sealed envelopes containing the group allocations. The randomisation materials will be locked in areas the blinded team cannot access. Once

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Healthy adults aged 18–50 years (inclusive)	
	Fluent spoken English—to ensure a comprehensive understanding of the research project and their proposed involvement	
	Capacity to provide written informed consent	
	Females of childbearing potential with a negative urine pregnancy test at screening and willing to practice adequate birth control measures during the study	
Exclusion criteria	Research participant:	Be currently involved in another study unless observational or non-interventional. Exceptions are the EHPC bronchoscopy study and COVID-19 observational and interventional trials. The exceptions will be applied at the discretion of the chief investigator to ensure no harm comes to the participants (eg, excessive blood sampling)
		Be a participant in a previous EHPC trial within the last 3 years (at the discretion of the study team)
	Previous pneumococcal vaccination:	
	Allergy:	Have allergy to penicillin or amoxicillin
		Have previous anaphylaxis or severe adverse reaction to any component/excipient of the vaccines or to any vaccine
	Health history (self-reported by the participant or confirmed in GPQ or medical summary if deemed necessary at clinician discretion): Ill health including but not limited to:	Asplenia or dysfunction of the spleen
		Chronic respiratory disease
		Chronic heart disease
		Chronic kidney disease
		Chronic liver disease
		Chronic neurological conditions
		Connective tissue disease
		Dementia
		Diabetes mellitus (inc. diet controlled)
		Immunosuppression or history of receiving immunosuppressive therapy
		Individuals with cochlear implants
		Individuals with major cerebrospinal fluid leaks
		Recurrent otitis media
	Have any uncontrolled medical/surgical conditions (such as but not restricted to mental health conditions, epilepsy, narcolepsy or chronic pain) at the discretion of the study doctor	
	Major pneumococcal illness requiring hospitalisation within the last 10 years	
	Other conditions considered by the clinical team as a concern for participant safety or integrity of the study	
	Medication:	That may affect the immune system, eg, steroids, inflammation altering (eg, nasal steroids, Roaccutane) or disease-modifying anti-rheumatoid drugs
		Long-term use of antibiotics
		Nitroglycerin
		That affects blood clotting (any oral/injectable anticoagulants (except aspirin))
	Female participants who are pregnant, lactating or intending on becoming pregnant during the study	
	Direct caring role or close contact with individuals at higher risk of infection (during the EHPC period):	Children under 5 years age
		Chronic ill health or immunosuppressed adults
		People that are part of the extremely vulnerable group as defined by Public Health England
	Smoker:	Current or ex-smoker (regular cigarettes/cigars/e-cigarette/vaping/smoking of recreational drugs) in the last 6 months
		Previous significant smoking history (more than 20 cigarettes per day for 20 years or the equivalent (>20 pack years))
	History or current drug or alcohol abuse (frequently drinking alcohol): men and women should not regularly drink >3 units/day and >2 units/day, respectively) at discretion of the clinician	
	Significant mental health problems (uncontrolled condition or previous admission in a psychiatric unit, at the discretion of the clinician) that would impair the participant's ability to participate in the study	
	Overseas travel planned: Overseas travel during the follow-up period Part A or Part B	
	Participants who meet STOP criteria at the time of screening, as detailed in table 3	
	Any other issue which, in the opinion of the study staff, may:	Put the participant or their contacts at risk because of participation in the study
		Adversely affect the interpretation of the study results
		Impair the participant's ability to participate in the study

Continued

Table 2 Continued

Temporary exclusion criteria	To inoculation:	Current illness and/or acute illness within 14 days of inoculation if COVID-19 negative
		Positive COVID-19 swab whether symptomatic or asymptomatic within 21 days of inoculation
		Currently isolating for 10 days following exposure to COVID-19
		Antibiotic use within 28 days of inoculation
	To vaccination:	Vaccination with an inactivated vaccine in the 14 days or with a live vaccine in the 28 days preceding study vaccination
		Vaccination with an approved COVID-19 vaccine in the 21 days preceding study vaccination
		Planned vaccinations during Part A or Part B of the study
		Current illness and/or acute illness including fever within 24 hours of vaccination if COVID-19 swab is negative
		Current illness and/or acute illness including fever within 21 days of vaccination if COVID-19 swab is positive
		Positive COVID-19 swab even if asymptomatic within 21 days of vaccination
		Currently isolating for 10 days following exposure to COVID-19
	Participants that have been temporarily excluded due to a positive COVID-19 swab, will require a negative lateral flow test prior to subsequent inoculation	

EHPC, experimental human pneumococcal challenge; GPQ, General Practitioner Questionnaire.

randomised, the used envelope and contents will be labelled with the participant study number, and this will provide an emergency unblinding point in case of eCRF failure (ie, in a medical emergency). Participants, the clinical and laboratory teams who will be processing samples, assessing participant safety and pneumococcal colonisation will be blinded to vaccine and strain allocation. Participants may wear a physical blindfold during the vaccine administration, which the unblinded clinical team will perform. The unblinded team performing vaccination can complete follow-up visits; however, they cannot perform NW samples or inoculations to avoid bias.

Day 7 visit post-vaccination

On day 7 post-vaccination, participants will attend for further sampling to enable systemic and mucosal immunological and transcriptomic analysis.

Re-screen

On day 23 post-vaccination, participants will attend for re-screening to confirm eligibility before inoculation. Study procedures will be the same as the screening visit except for the full blood count unless there is a clinical indication to repeat it.

Table 3 STOP criteria

Clinical history and examination	STOP if unexplained or concerning findings on history or examination
Engagement with research team	STOP if the research team have concerns about participant's ability to commit to frequent communication and safety checks
Illness during study	STOP if participant develops a medical condition or commences medication while on the study that would meet the exclusion criteria
Full blood count	STOP if Hb <10 g/L STOP if total WCC <1.5×10^9/L STOP if total WCC>12×10^9/L STOP if platelets<75×10^9/L
Resting SpO_2	STOP if <94%

Hb, Haemoglobin; WCC, White Cell Count.

EHPC

On day 28 post-vaccination, participants will attend the SPN3 inoculation visit. Ongoing informed consent and eligibility will be confirmed, and serum will be collected. The inoculum will be prepared as per the study laboratory manual. A mid-log broth culture of pneumococcus will be frozen at −80°C in aliquots of glycerol-enriched media. Frozen aliquots will be thawed, quantified for bacterial counts and purity before administration by a clinical team member intranasally as pre-specified in the clinical study manual. The participant will lie in a semi-recumbent position, and 0.1 mL of inoculum will be administered in each nostril with a pipette. The residual inoculum will be analysed to confirm the administered dose and purity.

Follow-up period

Participants will be followed-up for 23 days post EHPC. Microbiological (NWs), immunological (serum, PBMCs, nasal cells), transcriptomic (blood and nasal cells) and UAD (urine) samples will be collected during this period. If any symptoms potentially associated with the EHPC are reported, extra visits will be arranged for further assessment.

Part B

Part B of the trial follows the same visit and sampling schedule as part A. Participants will attend a re-screen at day 163, approximately 6 months post-vaccination, where eligibility will be reconfirmed. Inoculation in part B is with SPN6B.

Adverse events

Participants will be monitored for adverse events (AEs) at each visit following vaccination. For 5 days after EHPC, daily contact will be made before 12:00 to check for symptoms.

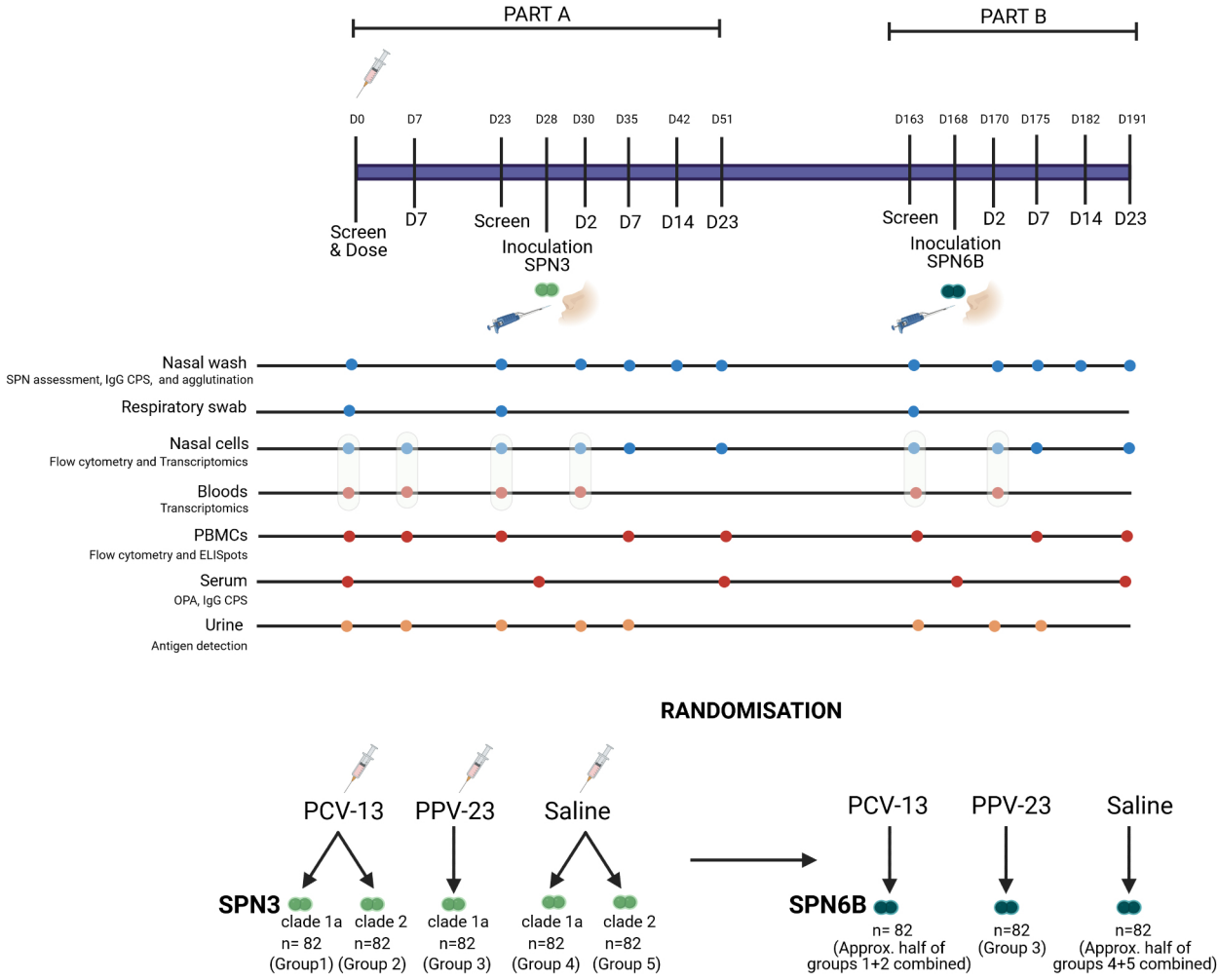

Figure 1 Study timeline (created with BioRender.com). CPS, capsular polysaccharide; OPA, opsonophagocytic activity; PBMC, peripheral blood mononuclear cell; PCV13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; SPN3, *Streptococcus pneumoniae* serotype 3; SPN6B, *Streptococcus pneumoniae* serotype 6B.

Safety

Participants are educated regarding participation risks and are closely monitored. We provide standby antibiotics to reduce time to treatment if required and 24-hour emergency telephone contact with researchers. We can facilitate access to hospital facilities if deemed necessary. Over the last 10 years of EHPC studies, we have had three separate serious AEs (SAEs) classified as unrelated to study protocols and conduct.

An independent Data and Safety Monitoring Committee (DSMC) will review safety and colonisation rate data throughout the study. All DSMC roles and responsibilities will be outlined in detail in the DSMC terms of reference. The committee's specific role will be:

- To independently review SAEs and adverse events of special interest (AESIs) regardless of relatedness to any study vaccinations or procedures throughout the study.
- To formally review the inoculum's safety profile and colonisation rate after the first 10 participants.
- To perform unscheduled reviews at the request of the study team at a frequency and demand determined by the severity of reported AEs/AESIs.

SPN3 challenge

Due to a perceived higher than usual proportion of participants exhibiting mild symptoms, particularly of pharyngitis, in our dose-finding study (Establishing SPN3 Challenge Model, unpublished data), we made amendments to continue to ensure the highest level of safety and evaluate further. We have introduced a questionnaire for 7 days post-inoculation that will measure symptoms objectively and compare them with previous EHPC studies. We will also compare the emergency antibiotic rates between SPN3 and SPN6B groups. An implemented standardised antibiotic prescribing algorithm eliminates any discrepancies in medical management between clinical team members.

Table 4 Study procedures during part A and B

Part A												Part B					
	Initial visit	**Screen (if separate)**	**Dose (if separate)**	**Screen and dose**	**Day 7**	**Re-screen**	**Inoculation (i) †**	**Day 2 (i+2)**	**Day 7 (i+7)**	**Day 14* (i+14)**	**Day 23 (i+23)**	**Re-screen**	**Inoculation (c) †**	**Day 2 (c+2)**	**Day 7 (c+7)**	**Day 14* (c+14)**	**Day 23 (c+23)**
Visit day/windows	D14 ±7	D5 +5/–7	D0	D0	D7 ±2	D23 +14/–2	D28 +14/–2	D30 ‡±1	D35 ‡±2	D42 ‡±5	D51 ‡±5	D163 +28/–14	D168 +28	D170 ‡±1	D175 ‡±2	D182 ‡±5	D191 ‡±5
Consent	X§	X§															
Clinical examination		X		X		X	X	X¶	X¶	X* ¶	X¶	X	X	X¶	X¶	X* ¶	X¶
Respiratory viral swab		X		X		X						X					
COVID-19 test		X		X	X¶	X		X¶	X¶	X¶	X¶	X		X¶	X¶	X¶	X¶
Vital signs		X	X	X		X						X					
Medical history	X	X		X													
Pregnancy test**		X**		X**		X**						X**					
GPQ sent if indicated	X¶																
Review symptoms					X	X	X	X	X	X*	X	X	X	X	X	X*	X
Randomisation			X	X													
Vaccination			X	X													
Inoculation							X						X				
Nasal wash‡‡		X		X		X		X	X	X*	X	X		X	X	X*	X
Nasal cells††			X††	X††	X††	X††		X††	X††		X††	X††		X††	X††		X††
Urine‡‡		X		X	X	X		X	X			X		X	X		
Blood (serum)‡‡			X	X			X		X††		X		X		X††		X
Blood (PBMCs)‡‡		X		X	X	X			X		X††	X			X		X††
Blood (transcriptomics)			X	X	X	X		X				X		X			
Blood full blood count‡‡	X§§	X§§										X					
Review AEs and SAEs					X	X	X	X	X	X*	X	X	X	X	X	X*	X
Symptoms questionnaire							X††	X††	X††	X††	X††		X††	X††	X††	X††	X††

Extra bacterial swabs may be taken for safety reasons or if participants develop symptoms during the trial.
*Attend visit if positive for pneumococcus in nasal wash by microbiological culture.
†Inoculation with SPN3 in part A (i) and inoculation with SPN6B in part B (c).
‡Visit day calculated from inoculation visit date in each part of the study, A and B. If inoculation delayed by n days, Day 2, 7, 14 and 23 (i/c+2, i/c+7, i/c+14, i/c+23) calculated from new inoculation visit date.
§Consent will be signed either at initial visit or screening visit if the former is via video or phone.
¶If clinically indicated.
**Women of childbearing potential.
††Subset of participants.
‡‡Additional samples may be taken, for example, to repeat a planned sample, on review of data or for safety reasons.
§§This sample will be collected in one of these visits.
AEs, adverse events; GPQ, General Practitioner Questionnaire; PBMC, peripheral blood mononuclear cells; SAE, serious adverse event; SPN3, *Streptococcus pneumoniae* serotype 3 ; SPN6B, *Streptococcus pneumoniae* serotype 6B.

Antibiotic therapy

Antibiotic therapy will be given to all participants after part A of the trial (amoxicillin 500 mg three times a day for 5 days) and to participants after part B who tested positive for SPN6B colonisation at any time point (amoxicillin 500 mg three times a day for 3 days). Emergency antibiotics may be given in certain circumstances post-inoculation:

1. Pharyngitis symptoms meeting the criteria for treatment as defined by the standardised algorithm.
2. Signs and symptoms consistent with pneumococcal disease at the discretion of the study team.

In these circumstances, the duration will depend on whether the participant is in part A or part B and the clinical need as assessed by the treating clinician.

Measurements

Colonisation

Colonisation will be determined by analysing NWs by classical quantitative culture and molecular methods. The rate of acquisition of colonisation will be compared between the intervention and control groups per serotype and clade. The density and duration of colonisation will also be measured by classical culture and molecular methods.

Immune measurements

Systemic and mucosal immune responses and their association with both acquisition and clearance of colonisation (density and duration) will be measured. We will collect blood samples (serum and PBMCs), NWs and nasal cells for this analysis.

Genetic measurements

Whole blood and nasal cells will be collected for RNA sequencing to determine gene expression and regulation in response to vaccination and challenge and potentially identify genetic factors that correlate with protection against acquisition of colonisation.

Viral detection and quantification

Detection and quantification of all common respiratory viruses will be performed by quantitative PCR on DNA and RNA extracted from respiratory swabs and NW.

Urine antigen detection

This limit assay test has been validated in adults,[48] and improves the specificity and sensitivity of identifying community-acquired VT-pneumococcal pneumonia.[49] The test's purpose will be to correlate against colonisation over time and density and refine the cut points used to define pneumococcal pneumonia versus colonisation in global studies.

Sample size

We aim for 410 participants to complete part A of the study to detect a 50% relative risk reduction in experimental SPN3 colonisation acquisition rates from 30% (detected by classical microbiology) in the control arm to 15% in the intervention (PCV13) arm. Each arm (figure 1, Groups 1+2 vs Groups 4+5) will need 164 participants to complete the study. The sample size has been calculated to achieve 96.2% power and a type I error (α) of 0.05. Up to 516 participants will be recruited to ensure 410 complete part A, allowing for a 20% dropout and screen failure rate from each group. Up to 312 participants will proceed to Part B to ensure 246 complete part A and B to detect a 50% relative risk reduction in experimental SPN6B colonisation acquisition rates (detected by classical microbiology) from 40% in the control arm to 20% in the intervention (PCV13) arm. Each arm (figure 1, 50% of Groups 1+2 vs 50% of Groups 4+5) will need 82 participants to complete the study. The sample size has been calculated to achieve 81.5% power and a type I error (α) of 0.05.

In calculating the sample size, we have assumed:

- SPN3 colonisation rates in unvaccinated adults aged 18–50 are 30%, as determined by classical microbiology from a recent EHPC study (Establishing SPN3 Challenge Model, unpublished data).
- SPN6B colonisation rates in unvaccinated adults are 40%, as determined by classical microbiology from previous EHPC studies.[41]
- PPV23 confers no protection against colonisation, as supported by Cochrane meta-analysis.[19] Therefore, we have not included this group in power calculations (figure 1, Group 3).
- PCV13 confers 50% relative risk reduction in colonisation against SPN3 as determined by microbiology compared with placebo at 1 month post-vaccination; 78% risk reduction against SPN6B colonisation seen in previous EHPC study.[41]
- A maximum of 10% screen failure rate and 10% dropout rates leading to non-completion.

During part A, unblinded team members will monitor colonisation rates in the control arm (figure 1, Groups 4+5). If colonisation is <30%, we will recalibrate the sample size accordingly, allowing for a 50% risk reduction between control and vaccine at 80% power and 5% significance level (table 5).

Interim analysis

After completion of planned enrolment and before the final analysis of part A of the study, we will reassess all sample size calculations inputs to determine if the sample size needs to be increased based on colonisation rates in the control arm and the risk reduction point estimate observed between control and vaccine. The sample size adjustments will be as per table 5. A nominal α (0.0025) will be assigned to this preliminary assessment. The remaining α (0.0475) will be used for the final analysis to ensure the overall hypothesis testing α is below the 0.05 significant level. If PCV13-associated risk reduction point estimates against SPN3 acquisition for both clades of SPN3 (combined and individual results) are <30%, additional enrolment will not be undertaken, as this is considered the limit of a meaningful outcome for the study.

Table 5 Sample size required per arm to detect a 50% relative risk reduction between intervention (PCV-13 SPN3 clades Ia and II) and control arms (saline SPN3 clades Ia and II) at 80% power and 5% significance level

Experimental SPN3 colonisation rates in control arm (%)	Sample size required per arm (50% risk reduction)	Sample size required per arm (40% risk reduction)	Sample size required per arm (30% risk reduction)
15	278	460	862
20	199	329	615
25	152	250	466
30	121	198	367
40	82	133	244
50	58	93	170
60	42	67	120

PCV13, 13-valent pneumococcal conjugate vaccine; SPN3, *Streptococcus pneumoniae* serotype 3.

Data analysis plan

Statisticians will perform statistical analysis using appropriate software. The intention-to-treat principle will be the main analysis strategy adopted for the primary and secondary outcomes. The membership of each analysis set will be determined and documented, and the reasons for exclusion given before database lock. A summary table will list the individual participants sorted by treatment group and describe their protocol deviation/violation. Detailed statistical analyses of primary and secondary outcomes will be described in the complete statistical analysis plan.

Patient and public involvement and engagement

A patient and public involvement and engagement group reviews the participant information leaflets and advertisement materials. The group will assist with advertising and recruitment planning, including public engagement events. We have numerous public and patient involvement opportunities: a newsletter sent out to inform of results and current studies. We also have social media accounts to update followers about trials and our ongoing work.

ETHICS AND DISSEMINATION

The study is approved by the NHS REC (Reference No:20/NW/0097) and the Medicines and Healthcare products Regulatory Agency (No:CTA25753/0001/001-0001). It is registered with ISRCTN and ClinicalTrials.gov.

Results will be published in a peer-reviewed journal and conferences when available. Publications will be consistent with the Consolidated Standards of Reporting Trials guidelines,[50] and based on the International Committee of Medical Journal Editors requirements.

LSTM's clinical trial insurance covers participants enrolled in the study for harm. The cover includes additional healthcare, compensation or damages.

Data access and management

The Trial Steering Group will have access to the data. If shared with collaborators outside LSTM, the data will be anonymised. Participant workbook documents will be locked securely in swipe card access spaces.

Study data will be collected and managed using Research Electronic Data Capture (REDCap) tools.[51 52] REDCap is a secure, web-based application designed to support data capture for research studies. After entry, data are quality controlled by a second staff member.

We will publicise a de-identified data set to an appropriate data archive within 3 years from study completion.

See Supplementary Appendix 2 for biological specimens statement.

LIMITATIONS

Recruitment in this trial will be limited to younger, healthy volunteers. In the EHPC model we expose participants to a pre-specified serotype at a single time point at a standard bacterial inoculum dose. This approximates natural exposure, but differs since natural exposure likely involves multiple exposures at various doses. Consequently, the trial results might not be fully generalisable to other populations such as young children, older adults or persons with comorbidities, and may not completely reflect the interaction between pneumococcal vaccines and natural exposure. Furthermore, it is possible that early antibiotic treatment requirement for AESIs or unexpected heterologous challenge protection might affect some secondary outcomes.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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