Guy's and St Thomas'
NHS Foundation Trust

CLINICAL STUDY REPORT

Vaccination for immune recovery following sepsis: The VACIRiSS Trial

Pneumococcal Vaccination to Accelerate Immune Recovery in Sepsis Survivors: randomized placebo-controlled trial

Sponsor Protocol Code:	VACIRISS, IRAS Project ID: 230431
EudraCT Number:	2017-002236-17
ClinicalTrials.gov Identifier:	NCT03565159
ISRCTN number:	230431
REC Reference:	18/EM/0079
Investigational Drugs (IMP):	Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed) [PCV13]
Indication:	Immunoprophylaxis in sepsis survivors
Development Phase:	Phase IV
Study Begin (FPFV):	1st August 2018
Study End (LPLV):	22nd April 2023
Report Version & Issue Date:	0.5
Sponsor Name and Address:	Guys and St Thomas' NHS Foundation Trust King's Health Partners Clinical Trial Office 16th Floor, Tower Wing, Guy's Hospital Great Maze Pond, London SE1 9RT
Chief Investigator:	Professor Manu Shankar-Hari

SIGNATURE PAGE

By signing below, I approve the contents of this Clinical Study Report, and confirm that to the best of my knowledge it accurately describes the conduct and results of the study. The clinical trial reported herein was conducted in accordance with the principles contained in the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable laws and regulations.

This was a non-commercial academic trial; the results of this study are not intended to be used for a licensing application.

Prof Manu Shankar-Hari

MSH

28-October-2025

Professor Manu Shankar-Hari, Chief Investigator (CI) Date

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APPENDICES

1. Ethics

1.1 Independent Ethics Committee

The study protocol and amendments were reviewed and approved by the East Midlands - Leicester Central Research Ethics Committee (REC).

1.2 Ethical conduct of the study

The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA)

1.3 Participant information and consent

Patients admitted to intensive care units or high dependency units with sepsis from 13 NHS tertiary centres in the UK, that were ready for step down care, were pre-screened for potential eligibility against the inclusion and exclusion criteria.

Potential participants were provided with the study specific REC approved patient information sheet (PIS). All approached potential participants, had the opportunity to review the PIS and ask any questions to the research team. Following the satisfactory resolution of any questions or concerns, written informed consent was secured from patients willing to participate.

2. Data Monitoring

2.1. Trial Steering Committee

A Trial Steering Committee (TSC) was established consisting of both independent and non-independent members including the Independent Chair. The TSC was an executive committee, responsible for the overall supervision of the trial on behalf of the Sponsor and Funder, and ensured that it was conducted in accordance with the rigorous standards set out in the UK Policy Framework for Health and Social Care Research and Guidelines for GCP. The TSC consisted of the CI and independent members composed of experienced critical care doctors, trialists, and Patient and Public Involvement (PPI) members. The TSC discussed any recommendations raised by the Data Monitoring Committee (DMC). The TSC meetings took place approximately two weeks following each DMC meeting including prior to start of participant recruitment, after the internal pilot stage, and at any other time that was determined by the Independent Chair.

2.2. Data Monitoring Committee

A DMC was appointed comprising two independent clinicians with experience in caring for and treating critically ill patients and an independent statistician. Biannual meetings were held, and recommendations made to the TSC. The DMC monitored recruitment, adverse events and outcome data. The DMC's responsibility was to safeguard the interests of the trial participants and advise the TSC to protect the validity and credibility of the trial. During the recruitment period, reports were provided to the DMC which included information on the AEs reported, all cause readmissions, death, recruitment, along with any other data that the committee requested. The DMC were advisory to the TSC. The DMC role would advise if, in their view, the randomised comparisons had provided both (i) 'proof beyond reasonable doubt' that for all, or some, the treatment is clearly indicated or clearly contra-indicated and (ii) evidence that might reasonably be expected to materially influence future patient management. Following a report from the DMC, the TSC decided any actions.

3. Sponsors, Investigators and Trial Sites

Sponsor

Guy's and St Thomas' NHS Foundation Trust

Chief Investigator

Professor Manu Shankar-Hari

Table 1: Investigators and Trial Sites

Site	Principal Investigator
Guy's and St Thomas' NHS Foundation Trust	Professor Manu Shankar-Hari (July 2018 – November 2021) Professor Marlies Ostermann (from November 2021)
King's College Hospital NHS Foundation Trust	Dr Philip Hopkins
University College London Hospitals NHS Foundation Trust	Dr David Brealey
Cambridge University Hospitals NHS Foundation Trust	Dr Charlotte Summers
NHS Lothian	Professor Timothy Walsh
Belfast Health and Social Care Trust	Professor Danny McAuley
Aneurin Bevan University Health Board	Professor Tamas Szakmany
Oxford University Hospitals NHS Foundation Trust	Dr Matthew Rowland (June 2019 – July 2022) Dr Stuart McKechnie (from July 2022)
Royal Surrey County Hospital NHS Foundation Trust	Dr Ben Creagh-Brown

Portsmouth Hospitals NHS Trust	Dr David Pogson
South Tyneside and Sunderland NHS Foundation Trust	Dr Anthony Rostron
Taunton and Somerset NHS Foundation Trust (Changed to Somerset NHS Foundation Trust)	Dr Richard Innes
Manchester University NHS Foundation Trust	Dr Tim Felton

4. Statistician, Laboratories, Database Management

Trial statistician

Professor David Harrison

Database Management

King's College London Clinical Trials Unit

Laboratories

King's College London

5. Study Synopsis

Title of clinical trial	Pneumococcal Vaccination to accelerate Immune Recovery in Sepsis Survivors: randomized placebo-controlled trial
Protocol Short Title/Acronym	Vaccination for immune recovery following sepsis - The VACIRiSS Trial
Study Phase	IV
Sponsor name	Guy's and St Thomas' NHS Foundation Trust
Chief Investigator	Professor Manu Shankar-Hari
Eudract number	2017-002236-17
REC number	18/EM/0079
IRAS project ID:	230431
Medical condition or disease under investigation	Sepsis

Purpose of clinical trial	The aim of VACIRiSS trial is to evaluate the immunogenicity and heterologous effects of single dose 13-valent conjugate pneumococcal vaccine (PCV-13) in preventing infection related rehospitalisation or death in sepsis survivors and to collect outcome event data with necessary precision to inform future definitive trial design
Primary objective	To compare the hazard ratio for infection related rehospitalisation or death within the 365-day follow-up period between intervention and control arm
Secondary objective (s)	- Derive outcome event data with necessary precision to inform future definitive trial design - To generate feasibility data and complete a focus group discussion during internal pilot phase of the trial - Describe immune recovery patterns in sepsis survivors - Describe vaccine responder characteristic
Trial Design	Randomised, double blind, placebo-controlled trial with internal pilot phase
Endpoints	Primary end-point: Differences in time to first infection related rehospitalisation or death within the 365-day follow-up period between the intervention and control arm Secondary end-points: - Generating precision estimates for the proportion of rehospitalisation, proportions of reinfections, proportion of reinfection related rehospitalisation, and time to first antibiotic therapy in general practice at different follow-up time points within the 365 days follow-up period between the intervention and control arm. Tertiary end-point: - Feasibility and qualitative thematic analysis outcome for pilot phase Exploratory endpoints of immune recovery patterns: - Differences in anti-pneumococcal antibody at baseline and 30 (+/-7) days between the intervention and control arm

	- Immune recovery patterns and heterologous vaccine effects will be measured at baseline (T0) before IMP administration, on 10 (+/-3) days post T0 in patients still in hospital, on 30 (+/-7) days' post T0 and 90(+/-7) days post T0. This will include flow cytometry (B cell subsets, T cell subsets and monocyte HLA-DR and PD-1 expression), function and leukocyte transcriptome differences between the intervention and control arm. - Define vaccine responder characteristic
Sample Size	N=214 with 107 in each arm
Summary of eligibility criteria	Patients who meet all the following inclusion criteria are eligible to participate in the trial. • Male or female adult patients aged 18 years or older on the date of screening for the trial • Registered with a General Practitioner • Reason for admission to intensive care unit or high dependence unit was sepsis • Clinical condition has improved, and the patient is ready for step down to HDU or ward based care in the next 24 – 48 hours • Provision of written informed consent by the patient OR by patient's Legal Representative OR professional consultee Patients who meet one or more of the following will be excluded from the trial. • Core temperature ≥38.0°C within the past 24 hours prior to study IMP administration. As with other vaccines, the administration of Prevenar 13 should be postponed in subjects suffering from acute, **severe** febrile illness. However, the presence of a **minor** infection, such as a cold, should not result in the deferral of vaccination. • Hypersensitivity reaction (e.g., anaphylaxis) to any component of Prevenar 13 or any diphtheria toxoid-containing vaccine.

	• Recent vaccination defined as any vaccination administered to subjects within 7 days of enrolment. • Pregnant and lactating women. • Limitations of care set including not for resuscitation, not for readmission to critical care. • Residence in a nursing home, long-term care facility, or other institution, or requirement of semiskilled nursing care. (An ambulatory subject who was a resident of a retirement home or village is eligible for the trial.) • As the IMP is administered intra muscularly, coagulopathy defined as platelet count less than 50 x 10^9/L and/or INR greater than 1.3. For this exclusion criteria bloods taken within 72 hours of screening are valid. If these standard of care blood results are not available, then these should form part of the screening bloods for assessing eligibility. • Splenectomy (previous or in the current admission) • Diagnosis of pneumococcal sepsis in the current admission • APACHE II score defined Immune deficiency or suppression, defined as presence of 1 or more of the following conditions: ▪ Documented human immunodeficiency virus (HIV) infection at any time-point pre-trial. If previous results are not available and/or current admission is not due to HIV infection, these patients do not need new testing and are considered eligible for the trial. ▪ leukaemia (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years) ▪ lymphoma (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)

	▪ Hodgkin disease (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years) ▪ multiple myeloma (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years) ▪ malignancy (defined as presence of any malignancy that had been treated by or had been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
IMP, dosage and route of administration	Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed) [PCV13], single dose (0.5mls), intra muscular
Active comparator product(s)	Equivalent volume (0.5mls) of sodium chloride 0.9% as placebo intervention
Maximum duration of treatment of a participant	Single IMP administration within 72 hours of randomisation (or when the patient is clinically well enough in the event of clinical deterioration post-randomisation) and 365-day follow-up. The last visit has a permissible window of +/-14 days for completion of final follow-up procedures.
Version and date of protocol amendments	V1.0 (04 January 2018) V1.1 (14 February 2018) V2.0 (27 March 2018) V3.0 (27 July 2018) V4.0 (29 October2018) V5.0 (15 Feb 2019) V6.0 (13 June 2019) V6.1 (22 February 2021) V6.2 (01 November 2021)

6. Glossary of terms

AE	Adverse Event
APACHE II	Acute Physiology and Chronic Health Evaluation II
APRIL	A proliferation-inducing ligand
AR	Adverse Reaction
AUC	Area under the curve
BAFF	B cell-activating factor
BCG	Bacillus Calmette-Guérin
BR1	Binding buffer 1
BSA	BD Pharmingen Stain buffer
BTMs	Blood transcriptomic modules
CD40L	Cluster of Differentiation Ligand
CGC	Cancer Genomics Cloud
CI	Chief Investigator
CRP	C-Reactive Protein
DEG	Differentially Expressed Genes
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
FCS	Flow Cytometry Standard
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GLM	Generalized Linear Models
GSIMP	Gibbs sampler-based left-censored missing value imputation
GSTT	Guy's and St Thomas' NHS Foundation Trust
HDU	High Dependency Unit
HES	Hospital Episode Statistics
HIP	Human Immunology Project
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HRQoL	Health-related quality of life (HRQoL)
ICD-10	International Classification of Diseases
ICU	Intensive Care Unit
IE	Intercurrent Event
IgG	Immunoglobulin-G
IMP	Investigational Medicinal Product
IRAS	Integrated Research Application System
IRD	Incidence Rate Difference

IRR	Incidence Rate Ratio
ISRCTN	International Standard Randomised Controlled Trials Number
KCL	King's College London
KCTU	King's College Clinical Trials Unit
LPLV	Last Patient Last Visit
LRT	Likelihood Ratio Test
MedRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
PBMC	Peripheral Blood Mononuclear Cell
PCV13	Pneumococcal polysaccharide conjugate vaccine (13-valent, adsorbed)
PI	Principal Investigator
PIS	Participant Information Sheet
PPI	Patient Public Involvement
PT	Processing Tube
REC	Research Ethics Committee
RPMI	Rosewell Park memorial Institute medium
RR	Risk Ratio
SAE	Serious Adverse Event
SAR	Serious Adverse Reactions
SLEA	Sample-Level Enrichment Analysis
SOFA	Sequential Organ Failure Assessment
SSIP	Sepsis Survivor Prognosis Score
STM	Science Translational Medicine
TSC	Trial Steering Committee
UK	United Kingdom
VST	Variance Stabilized Transformed
WHO	World Health Organization

7. Publication (reference)

We have submitted the manuscript to the Science Translational Medicine (STM) – currently awaiting decision.

8. Study period (years)

The study ran for 4 years and 8 months.

8.1 Table 2: Recruitment Milestones and Dates

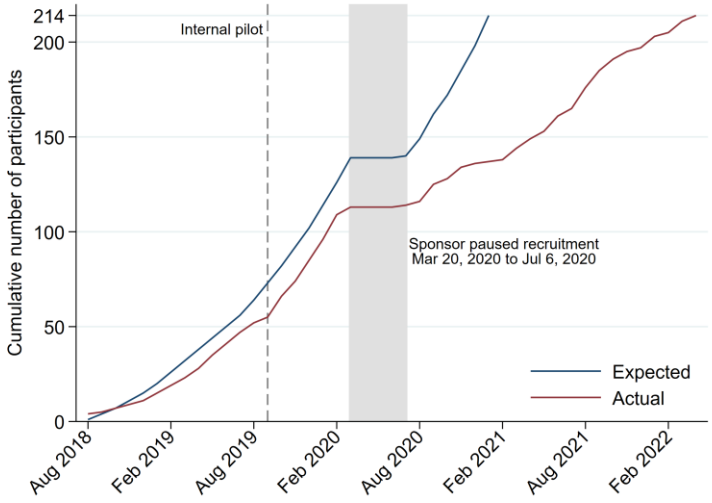
Recruitment Milestones	Date
Open to recruitment	27th July 2018
First Patient First Visit (FPFV)	1st August 2018
Completion of internal pilot*	25th September 2019
Recruitment paused	20th March 2020
Recruitment restart	6th July 2020
Last patient randomized	22nd April 2022
Last Patient Last Visit (LPLV)	22nd April 2023

*Note: The internal pilot phase (n=54 participants) included a focus group with clinicians and patients (not trial participants) on acceptability of trial design, and a data monitoring and safety committee meeting, which recommended continuing the trial without modifications.

8.2 Impact of COVID-19 pandemic on the trial

Recruitment was halted by the Sponsor on 20th March 2020 until 6th July 2020 coinciding with the first wave of the COVID-19 pandemic. Each site then underwent a capacity and capability assessment to restart recruitment. Of the 13 sites, 10 had reopened to recruitment by the end of August 2020. However, stretched resources resulted in slow recruitment. Although no further pauses to recruitment were enforced by the trial Sponsor (as per UK National guidance), several sites intermittently paused to recruitment between 1st December 2020 and 28th February 2021. See timelines figure below.

Figure 1: Recruitment Timelines



8.3 End of trial

The end of the trial is defined as database lock, following completion of analysis of biological samples for exploratory outcomes, addition of results to the electronic Case Report Form (eCRF) and following resolution of queries.

9. Phase of development

IV

10. Objectives

10.1 Primary objective

To compare the hazard ratio for infection related rehospitalisation or death within the 365-day follow-up period between intervention and control arm

10.2 Secondary objectives

There are four secondary objectives:

- Derive outcome event data with necessary precision to inform future definitive trial design
- To generate feasibility data and complete a focus group discussion during internal pilot phase of the trial
- Describe immune recovery patterns in sepsis survivors
- Describe vaccine responder characteristic

11. Background and Context

Sepsis is defined as life-threatening acute organ dysfunction caused by dysregulated host responses to infection (*1*). Globally, sepsis is common and deadly, with extrapolated annual incidence and deaths estimated at ~48 million and ~12 million, respectively (*2*). Thus, ~36 million people survive sepsis hospitalizations (referred as sepsis survivors hereon) every year. Sepsis survivors frequently suffer from long-term ill health (*3, 4*). Among adults who survive sepsis-related critical illness, ~45% are hospitalized and ~15% die within the first year following hospital discharge (*3, 5, 6*). Improving their long-term ill health is an unmet clinical need (*7, 8*). Multiple quantitative and functional immune impairments described in human sepsis survivor cohorts and animal models (*9-12*) result from the residual effects of dysregulated immune responses during sepsis (*13-15*) (such as lymphocyte apoptosis (*16, 17*), innate and adaptive immune cell dysfunction, persistent inflammation), could increase the risk of infection-related rehospitalizations in sepsis survivors (*18*). Although some of these immune impairments are

potentially modifiable with vaccines (*19*), vaccine immunogenicity has never been systematically examined in sepsis survivors. The putative T cell impairments in sepsis survivors risks uncontrolled pathogen replication (*20*) precluded us from using Bacille-Calmette Guerin (BCG) or other live attenuated vaccines with established innate immune heterologous effects (*21*- 19 *23*), as the intervention to test in the first clinical trial of vaccines in sepsis survivors. This led us to consider adjuvant vaccines with T-cell dependent B cell responses to test in such a trial, as that vaccine would give readouts on T cell function and antigen presentation in sepsis survivors, alongside the non-specific immune responses from the adjuvant. Given concurrent acute inflammation and immunosuppression in sepsis survivors, we exercised caution and avoided directly extrapolating data on a conjugate vaccine's immunogenicity, efficacy, and safety evidence from immunosuppressed high-risk populations with cancer or chemotherapy (*24*). Additionally, concurrent acute inflammation and immunosuppression in sepsis survivors adds uncertainty to the choice of immunomodulators as an intervention. At the time of designing our trial, guidelines on vaccinating immunocompromised patients consistently recommended pneumococcal and seasonal inactivated influenza vaccines (*25, 26*). These observations formed our rationale for choosing 13-valent pneumococcal vaccine conjugated to CRM197 carrier protein, adsorbed on aluminium phosphate (PCV13; Pfizer) as the intervention to test in this trial, as PCV13 immunogenicity involves T cell-dependent activation of B cells (*27, 28*) along with adjuvant mediated non-specific effects from activation of innate leukocytes and lymphocytes (*23*), without the putative risks of live vaccines in this population (*20*). In this context, the Pneumococcal Vaccination to Accelerate Immune Recovery in Sepsis Survivors (VACIRiSS) trial tested the hypothesis that, compared with placebo, a single intramuscular dose of PCV13 in adult sepsis survivors (n=214 participants) would improve the composite outcome of time to first infection-related rehospitalization or death at 365 days, and the immunogenicity hypothesis being that we will observe serotype-specific IgG responses alongside additional non-specific responses at whole blood transcriptome, cytokines and immune cell subsets levels. We report that PCV13 induced serotype-specific IgG responses without concomitant improvements in clinical outcomes. We observed biologically plausible non-specific effects within blood transcriptomic modules (BTMs), changes in cytokines and in immune cell subsets. PCV13 immunogenicity in sepsis survivors generated two of the three previously reported pre-vaccination innate immune inflammatory endotypes (*29*). Additionally, the previously reported baseline predictors of PCV13 responsiveness, such as male gender and cytotoxicity-associated genes (*30*) differed by the pre-vaccination innate immune inflammatory endotypes (*29*) and explained the observed biological variation in PCV13 responses.

12. Methodology

12.1 Trial design and oversight

The Pneumococcal Vaccination to Accelerate Immune Recovery in Sepsis Survivors (VACIRiSS) trial was a multicenter, placebo-controlled randomized trial that enrolled participants from 13 adult general critical care units (ICUs) in National Health Service (NHS) hospitals in the UK. The trial

protocol was approved by the Leicester Central Research Ethics Committee (Ref:18/EM/0079). Clinical trial authorization was provided by the Medicines and Healthcare Products Regulation Agency. Site investigators, or trained delegates, assessed the eligibility of potential participants. Informed consent was provided by the participants or their legal representatives. Additional details appear in the trial protocol.

12.2 Participants

Eligible participants were adults (≥18 years of age) admitted to ICU with sepsis, whose clinical condition had improved and were ready for general ward concentration care. Sepsis was defined as clinically suspected or proven infection with evidence of organ dysfunction measured using the Sequential Organ Failure Assessment (SOFA) score (1). Exclusion criteria included splenectomy, pneumococcal sepsis, Acute Physiology and Chronic Health Evaluation II (APACHE II)-defined immunodeficiency or suppression, coagulopathy precluding intramuscular injection, pregnancy, vaccinations in the last 7 days, core temperature >38°C in the last 24 hours, or expected death or withdrawal of life-sustaining therapy within 48 hours. Details of the eligibility criteria are provided in section 14.

12.3 Randomization, masking and procedures

Participants were randomly allocated 1:1 to a single intramuscular dose of either PCV13 or placebo, stratified by site and age (≤65 vs >65 years based on UK vaccination guidance (60)), with a password-protected, encrypted, centralized web-based system using permuted blocks of variable size. Participants, site research staff, those administering the intervention, statisticians and personnel performing follow-up were blinded to the allocated intervention. Each trial site had independent unblinded teams to dispense the allocated intervention. All other aspects of care were at the discretion of treating teams.

12.4 Follow-up

Randomization was baseline (T0). In-person follow-up time-points for outcomes were T0, 10(±3)days (T1) 30(±7)days (T2) and 90(±7)days (T3). Telephone follow-up occurred at 90(±7)days (T3), 180(±7)days (T4) and 365(±14)days (T5). Trial-specific data were linked with routine data to supplement follow-up as described in the 'Linkage to routine data sources' section below.

Table 3: Schedule of trial assessments

Procedures	Screening within 5 days post consent	T0 Baseline (within 72hrs of randomisation)	Follow-up visits days post-IMP administration in days				
			T1[11] 10 (+/-3) days	T2 30 (+/-7) days	T3 90 (+/-7) days	T4 180 (+/-7) days	T5 365 (+/-14) days
Written informed consent	X						
Demographics	X						

Procedures	Screening within 5 days post consent	T0 Baseline (within 72hrs of randomisation)	Follow-up visits days post-IMP administration in days				
			T1[11] 10 (+/-3) days	T2 30 (+/-7) days	T3 90 (+/-7) days	T4 180 (+/-7) days	T5 365 (+/-14) days
Medical history	X						
Pregnancy test for women	X						
Index sepsis characteristics	X						
Eligibility assessment	X	X					
Randomisation	X						
Bloods for FBC and CRP		X	X	X	X		
Research blood sampling		X	X	X	X		
Physical examination		X	X	X	X		
Concomitant medications	X	X	X	X	X	X	X
IMP administration		X					
Dispense Vaccine diary		X					
Adverse event monitoring		X	X	X	X	X	X
HRQOL questionnaires		X		X	X	X	X
Arrange follow up visits		X					
Collect vaccine diary			X				

12.4.1 Linkage to routine data sources

(a) ICNARC Case Mix Programme

Trial-specific data for participants recruited from sites in England, Wales and Northern Ireland were linked at the individual patient level with data from the ICNARC Case Mix Programme, the national clinical audit of adult critical care. Data obtained from the Case Mix Programme included:

- Quintile of deprivation, for use in the Sepsis Survivor Prognosis Score (SSIP) score (see 17.5)
- Additional values for baseline data from the index critical care unit admission, where data were missing from the Case Report Form
- Patient identifiers for onward linkage to routine data held by NHS England

As the Case Mix Programme does not cover critical care units in Scotland, the one site in Scotland provided deprivation data directly as an additional data upload.

(b) NHS England

Patient identifiers (NHS Number, date of birth, sex) for participants recruited from sites in England and Wales were passed to the NHS England Data Access Request Service for linkage with Hospital Episode Statistics Admitted Patient Care and Demographics datasets. Data obtained from NHS England included:

- Dates of all acute hospital admissions to NHS hospitals in England during the year

preceding randomization, to allow calculation of the number of previous hospitalizations for the SSIP score (see 17.5)

- Dates and International Classification of Diseases (ICD-10) diagnostic codes for all acute hospital admissions to NHS hospitals in England during the year following randomization, for calculation of the primary and secondary outcomes
- Dates of death, for cross-checking with dates reported via the trial case report form

As NHS England Hospital Episodes Statistics only cover hospitals in England, sites in Scotland, Wales and Northern Ireland confirmed the above information via local systems and reported it either via the trial case report form or by additional data uploads.

12.5 Outcomes

12.5.1 Clinical outcomes

The estimands framework (*61*) for the primary outcome was as follows:

Treatment	Single dose of pneumococcal conjugate vaccine 13 compared with placebo
Population	Adult sepsis survivors (Eligibility criteria as below in section 14 of this report; Section 4.2 of Protocol)
Primary outcome	time to first infection related rehospitalization or death within 365 days of randomization (Section 5.1 of Protocol)
Intercurrent events (IE)	Death as an IE for infection related rehospitalization has been handled using a composite strategy. Specific IEs, which will apply across intervention and control arms, and which will be handled using a treatment policy strategy are: (a) withdrawal from treatment for safety reasons; (b) COVID-19 infection
Population level summary	Incidence rate ratio (IRR) and incidence rate difference (IRD)

The detailed definitions of all the reported clinical outcomes are provided in the sub sections below. Briefly, the primary outcome was time to first infection-related rehospitalization or death due to any cause in the 365-day follow-up period. Dates of hospitalization and deaths were obtained from data linkage with Hospital Episode Statistics (HES) where an ICD-10 code indicated infection as the cause for admission and cross-checked with the trial case report form. HES is a curated data product containing details about admissions and attendances at NHS hospitals in England (*62*). Secondary outcomes included binary outcomes of all-cause rehospitalization, reinfections and infection-related rehospitalization, and time-to-event outcomes of first all-cause rehospitalization and first antibiotic therapy in general practice. Health-related quality of life (HRQoL) was an exploratory clinical outcome.

a) Primary clinical outcome (endpoint)

The primary outcome is time to first infection related rehospitalization or death within the 365 days follow-up period from randomization, defined as the earlier of the date of death or date of infection related hospitalization minus the date of randomization plus one.

Date of death may be obtained from:

i. The trial case report form – from the Completion/Withdrawal form, the date of withdrawal (within 365 days following randomization) where the reason for withdrawal is recorded as "death of participant"

ii. Data linkage with Demographics data – the date of death (within 365 days following randomization) from a death registered with the Office for National Statistics

Date of first infection related hospitalization may be obtained from:

i. The trial case report form – from the Rehospitalization Form completed at each follow-up visit, the earliest date of admission to hospital (within 365 days following randomization) for which the patient (or their relative/next of kin or GP) has indicated that they were given antibiotics for infection during the admission

ii. Data linkage with Hospital Episode Statistics – the earliest date of admission to hospital (within 365 days following randomization) indicating the start of a hospital admission during which an ICD-10 code indicating infection (identified by mapping ICD-10 codes to Healthcare Cost and Utilization Project Clinical Classifications Software multilevel categories was reported as the primary diagnosis within any hospital episode during the admission. In our previous publication, that performed derivation and validation of this approach to rehospitalization outcomes.

As the primary outcome is a composite of two events, the two separate components – time to first infection related rehospitalization and time to death – will also be reported separately. For analyses of time to first infection-related hospitalization, patients that die without the event will be censored at the date of death.

Any conflict between the data sources, data linkage with civil registrations for mortality and hospital episode statistics for rehospitalization outcome will take precedence.

b) Secondary clinical outcomes (endpoints)

1. Rehospitalization

Rehospitalization will be assessed as a binary outcome (rehospitalized or not rehospitalized) at 30-, 90-, 180- and 365-days following randomization, defined as the date of first rehospitalization being prior to or equal to the date of randomization plus 30, 90, 180 or 365 days. Date of first hospitalization may be obtained from:

i. The trial case report form – from the Rehospitalization Form completed at each follow-up visit, the earliest date of admission to hospital recorded

ii. Data linkage with Hospital Episode Statistics – the earliest date of admission to hospital (within 365 days following randomization).

Any conflict between the data sources, data linkage with civil registrations for mortality and

hospital episode statistics for rehospitalization outcome will take precedence.

2. Reinfection:
Reinfection will be assessed as a binary outcome (reinfected or not reinfected) at 30-, 90-, 180- and 365-days following randomization, defined as prescription of antibiotics from concomitant medication or from the Serious Adverse Event (SAE) log.

3. Infection related rehospitalization
Infection related rehospitalization will be assessed as a binary outcome (infection related rehospitalization or no infection related rehospitalization) at 30-, 90-, 180- and 365-days following randomization, defined as the date of first infection related rehospitalization being prior to or equal to the date of randomization plus 30, 90, 180 or 365 days. Date of first infection related rehospitalization will be defined as for the primary outcome.

4. Time to first all cause rehospitalization
Time to first all cause rehospitalization will be assessed within the 365-day follow-up period from randomization, defined as the date of first rehospitalization minus the date of randomization plus one. Date of first rehospitalization will be defined as for the rehospitalization outcome.

5. Time to first infection requiring antibiotic therapy
Time to first antibiotic therapy in general practice will be assessed within the 365-day follow-up period from randomization, defined as the date of first infection requiring antibiotic therapy minus the date of randomization plus one. Date of first infection requiring antibiotic therapy will be defined using the date recorded in the concomitant medication log or from the General practitioner.

c) Exploratory clinical outcomes (endpoints)
6. Health-related quality of life
Health-related quality of life will be assessed using E5-5D-5L questionnaires administered in person or via phone, at baseline, at 30-, 90-, 180- and 365-days following randomization, with baseline defined as the date of randomization. EQ-5D-5L responses will be valued using the NICE Decision Support Unit (EEPRU) mapping from EQ-5D-5L to EQ-5D-3L43 to generate an index score, anchored at 0 (dead) and 1 (perfect health). Patients known to have died at any timepoint will be assigned a value of 0.

12.5.2 Biological Outcomes
All biological outcomes were considered exploratory and assessed using blood samples collected and stored at -80°C, with all measurements performed blinded to treatment allocation.

a) Measurement of strain specific anti-pneumococcal immunoglobulin G (IgG)
Vaccine immunogenicity was assessed using serotype-specific immunoglobulin-G (IgG) to the 13 serotypes of Streptococcus pneumoniae (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 24 and 23F), at T0 and T2 to coincide with peak IgG responses (*34*). These were assayed at the World Health

Organization (WHO) reference Laboratory for Pneumococcal Serology based at the Institute of Child Health, University College London. IgG concentrations to capsular polysaccharides of all vaccine serotypes were measured in serum using the WHO standardized enzyme-linked immunosorbent assay (ELISA) method, following adsorption with cell wall polysaccharide and 22F polysaccharide at a concentration of 10 mg/mL (*54*). ELISA plates were coated with 5 µg/mL of each purified CPS (Statens Serum Institut). Serum samples were used in three 1:3 serial dilutions, starting from 1:50. Antigen-specific antibodies were detected by goat anti-human IgG (1/4000; Fc-specific) al-kaline phosphatase (Sigma). Optical density was measured at 405 nm using FLUOstar Omega plate reader (BMG 35 Labtech).

b) Targeted cytokines profiles using Legendplex™ (BioLegend) assay

In a subset of participants, cytokines, and lymphocyte subsets were measured at T0, T1, and T2. Cytokines measured included B cell-activating factor (BAFF), a proliferation-inducing ligand (APRIL), cluster of differentiation ligand (CD40L), interleukins (IL-6, IL-17A, IL-10) and chemokines (CXCL8, CXCL10). Patients undergoing elective cardiac surgery acted as age-matched healthy controls (ethics approval:19/SC/0187)(44).

Cytokines were measured using Legendplex™ (BioLegend) bead-based multiplex assays. Beads, detection antibodies and Streptavidin-phycoerythrin (SA-PE) were diluted 2-fold in assay buffer. Matrix B or assay buffer was added to each well in a 96-well V-bottom plate prior to the addition of serum samples or standards. 12.5 µl of sample and 25 µl of standard was added to respective wells resulting in a 2-fold and 4-fold dilution, respectively. The pre-mixed beads were vortexed for 1 minute and 25 µl of mixed beads were added to each well, vortexing beads intermittently to avoid bead settling. The plate was placed on a plate shaker at room temperature at 600 RPM for 1.5 hours. The plate was centrifuged at 250 xg for 5 minutes to pellet the capture beads bound to analytes. The supernatant was removed and washed twice in the wash buffer. 25 µl of biotinylated detection antibody was added to each well and incubated on a plate shaker at room temperature at 600 RPM for 45 minutes. SA-PE was subsequently added to each well and incubated for 30 minutes on the plate shaker at room temperature at 600 RPM. The plate was centrifuged and washed twice in the wash buffer before being resuspended in 100 µl of wash buffer for analysis. Samples were acquired on a BD LSR Fortessa flow cytometer. Bead population was gated on, and 4400 events were acquired per standard/sample gating to achieve the recommended 300 events per analyte. Flow Cytometry Standard (FCS) files were analyzed using LEGENDplexTM Data Analysis Software Suite (64).

c) Leukocyte phenotyping

Isolation and storage of peripheral blood mononuclear cells

Blood samples were collected from patients at timepoints, (T0, T1, T2) and processed for Peripheral Blood Mononuclear Cell (PBMC) isolation using BD Vacutainer® CPT™ tubes. Samples were first inverted 10 times before being centrifuged at 1800g for 25 minutes at room temperature. CPT tubes were inverted to mix PBMCs with the plasma layer. The mixture of plasma and PBMCs were transferred directly to a 50ml falcon tube and cell culture media (Rosewell park memorial institute

medium (RPMI)) 1640, 10% Fetal bovine serum (FBS), 5% penstrep) was added to a total of 50mls. Samples were centrifuged at 500g for 5 minutes, resuspended in 1ml of cell culture media and counted using countess 3 automated cell counter. Samples were then resuspended to a concentration of 1x107/ml in freezing media (FBS and 5% DMSO) and 1 ml was aliquoted into cryovials. Cryovials were placed in refrigerated Mr frosty and frozen at -80°C.

Immune cell phenotyping panels

We designed two different flow cytometry panels for standardized immunophenotyping of T and B lymphocyte subsets, per the Human Immunology Project (42). The T cell panel consisted of the following targets (fluorochrome; clone; catalogue number; antibody volume in µl per 100 µl): Fixable Viability stain 780 (APC-Cy7; FVS780; 565388; 0.2); CD3 (BUV395; SK7; 564001; 2.0); CD4 (BV786; SK3; 563877; 2.0); CD8 (BV605; SK1; 564116; 2.0); CCR6 (CD196) (BB515; 11A9; 564479; 2.0); CXCR3 (CD183) (BB700; 1C6; 566532; 2.0); CCR7 (CD197) (BV421;); CD45RA (APC; HI 100; 550855; 5.0); CD25 (BV510; 2A3; 740198; 2.0); CD127 (BUV737; HIL-7R-M21; 612794; 2.0); CCR4 (CD194) (PE-CF594; 1G1; 565391; 2.0) and CD45RO (PE; UCHL1; 555493; 5.0). The B cell panel consisted of the following targets (fluorochrome; clone; catalogue number; antibody volume in µl per 100 µl): Fixable Viability stain 780 (APC-Cy7; FVS780; 565388; 0.2); CD19 (BV711; SJ25C1; 563038; 2.0); CD27 (BV786; L128; 563327; 2.0); CD43 (BV421; 1G10; 562916; 2.0); CD24 (BUV395; ML5; 563818; 2.0); IgM (BB515; G20-127; 563327; 2.0); IgG (APC; G46-6; 550931; 2.0); IgD (BUV737; IA6-2; 612798; 2.0); and CD38 (PE; HIT-2; 555460; 5.0).

Flow cytometry and acquisition

All flow cytometry antibodies and concentrations used for analysis can be found below. PBMC samples were stained for viability with BD Horizon Fixable Viability Stain 780 for 10 min at room temperature in PBS. PBMC staining was performed in BD Pharmingen Stain buffer (BSA) and BD Horizon Brilliant Stain buffer Plus for 20 min at room temperature. For B and T cell panels, 100 µl of sample was analyzed on a five-laser BD LSR Fortessa acquired with a BD high-throughput sampler.

d) Panleukocyte transcriptome

Whole blood was collected from patients at each sample into a PaxgeneRNA (BD) tube for RNA extraction, Ethylenediaminetetraacetic acid (EDTA) plasma tube for plasma isolation. PaxgeneRNA tube was left to stand for 2 hours at room temperature and then stored in -80°C until extraction of RNA. EDTA plasma tube was centrifuged at 1300g for 10 minutes and plasma was aliquoted and stored at -80°C.

Whole blood RNA-seq

PaxgeneRNA tubes were processed in an automated fashion using the Paxgene blood RNA kit (IVD) (Qiagen 762174) and the Qiacube (Qiagen). PaxgeneRNA tubes were thawed in batches of 12. Where possible all of one patient's samples were extracted in the same batch. PaxgeneRNA tubes were placed in room temperature for two hours to thaw. PaxgeneRNA tubes were centrifuged (Settings: 10 minutes, 3000g, room temperature). Supernatant was discarded, blotted on clean paper towel and the pellet was resuspended in 4ml RNase-Free water. A new secondary BD

hemograd lid was fitted and the tube was vortexed until pellet was resuspended. Tubes were centrifuged once more at the same settings. All supernatant was removed, and the pellet was resuspended in 350µl of Binding buffer 1 (BR1), vortexed until dissolved and pipetted into a processing tube (PT). Protocol A and protocol B were followed as per manufacturer's protocol (*65*). Extracted RNA was stored in -80°C. Quantity of RNA was measured using Qbit.

RNA library preparation and sequencing

Library preparation and sequencing was conducted by Genewiz (Azenta) in three batches. For each sample 20µl of RNA at a concentration of 50ng/µl resulting in a total amount of RNA of 1000ng was delivered to Genewiz. Libraries were created using NEBNext Ultra II, PolyA selection directional RNA library prep kit alongside Fast Select Globulin depletion kit and ERCC spike in mix. Sequencing was performed using Illumina Novaseq system with a 2X150bp configuration. Data output was an estimated 20-30million raw paired-end reads. Data was returned in FASTQ format for alignment in house.

e) Analysis of Biological Data

Analysis of cytokine data

FCS files were uploaded to the LEGENDplex™ analysis software suite which is an automatic analysis cloud-based software. The software calculates standard curves for each analyte and extrapolates sample concentrations from PE median intensity. Data was outputted as a CSV and loaded into the R statistical environment for data visualization. We then used Gibbs sampler-based left-censored missing value imputation approach (GSIMP) to impute values for samples which fell below the limits of detection. Cytokines with missing values ≥30% were excluded **(Fig.A1)**.

Analysis of Immune cell phenotyping data

FCS files were analyzed using FlowJo (v.10.10). Gating strategies for all panels are outlined in Supplementary (**Fig.A1** shows T cell gating approach and **Fig.A2** shows B cell gating approach). Frequencies of relevant populations were calculated in R. For the B cell panel, samples with under 1000 CD19+ cells were excluded.

RNAseq analysis Alignment

FASTQ files were uploaded on the Cancer Genomics Cloud (CGC) (*66*), a high-performance computer cluster tool for bioinformatics. Within the CGC, FASTQ files were trimmed to remove adapter content using Trimmomatic (*67*) and aligned to genome reference consortium human build (GRCh38) using salmon (*68*). Quality of alignment was assessed using multiQC (*69*). The proportion of aligned reads and number of aligned reads was acceptable. The output of files was downloaded onto a local computer for further analyses.

Differentially expressed genes

Quant files, outputted by salmon, were brought into the R statistical environment for differential expression analysis in Deseq2 (*50*) via tximport (*70*). Deseq2 fitted generalized linear models (GLM) for each gene to compare contrasts of; PCV13 T0 vs Placebo T0, PCV13 T1 vs Placebo T1, PCV13 T2 vs

Placebo T2, responders T0 vs non-responders T0, responders T1 vs non-responders T1, responders T2 vs non-responders T2. To account for batches in the dataset, sequencing batches were encoded as a fixed effect. To gain a greater insight into molecular pathways identified in the analyses the gene count matrix from Deseq2 was used in Qusage (*53*) determine differential expression of gene collections (modules) from the blood transcriptional modules (*71*) and the Hallmark modules from MSigDB (*72*). We then performed the same comparisons as described above.

Pre-vaccination innate immune endotypes (29)

For identifying pre-vaccination innate immune endotypes, we followed the approach proposed by Fourati and Colleagues. Briefly, sample-level enrichment analysis (SLEA) gene expression data were variance stabilized transformed (VST), and batch corrected using Limma v3.60.6. Baseline (T0) sample expression values were median-centered and standardized by dividing by standard deviation. SLEA was conducted using Gitools v2.3.1 to estimate z-scores for each blood transcript module (BTM) and hallmark gene sets across baseline samples. Selection of BTM and hallmark gene sets were used for SLEA, as described previously. Hierarchical clustering was performed using Euclidean distance and complete linkage. The optimal number of clusters was determined using NbClust v3.0.1. Each sample was designated an inflammatory tier based on the average SLEA z-score of four hallmark gene sets (HALLMARK_INFLAMMATORY_RESPONSE, HALLMARK_COMPLEMENT, HALLMARK_IL6_JAK_STAT3_SIGNALING and HALLMARK_TNFA_SIGNALING_VIA_NFKB).

13. Number of patients (planned and analysed)

13.1 Planned

Sample size

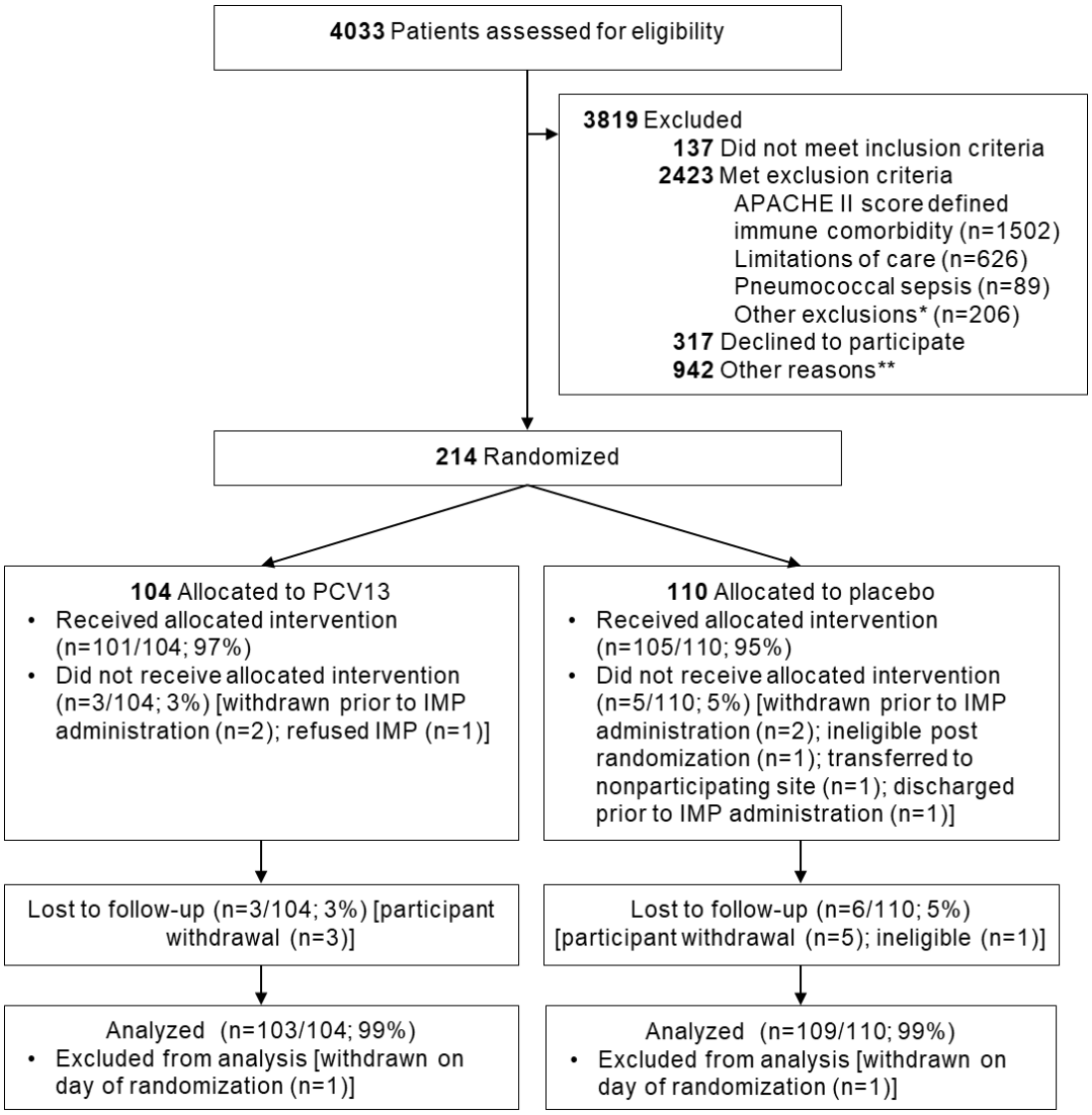
Our systematic reviews (5, 6), highlighted an expected rate for infection-related hospitalization or death by 365 days of 32.1%. No prior information was available on expected treatment effect for PCV13 in sepsis survivors. A sample size of 214 would provide 90% power to detect a hazard ratio (HR) of 0.36 for the primary outcome compared with PCV13 with placebo ($P<0.05$).

13.2 Analysed

Between July 27, 2018, and April 22, 2022, 4033 adult sepsis survivors were assessed for eligibility. 214 participants were randomized, with 104 allocated to PCV13 and 110 to placebo. Of these, 2.9% (3/104) allocated to PCV13 and 4.5% (5/110) allocated to placebo did not receive intervention. One participant from each group was withdrawn on the day of randomization **(Fig.2)**. The PCV13 and placebo groups had largely comparable baseline characteristics **(Table 6)**, although there were higher proportions of medical admissions and dependency in the placebo group as well as differences in primary site of infections between PCV13 and placebo groups. We report the features of the sepsis survivor population in UK ICUs (i.e., trial setting) over the trial recruitment period **(Table-S1)**. This highlights that the trial population is representative of the sepsis survivor population in the UK, albeit the trial population was marginally older, with greater severity of illness, and higher proportion of participants with self-reported ethnicity as White. Study specific follow-up was

completed on April 22, 2023.

Figure 2: **Flow diagram showing Screening, Randomization, and Analysis of Study Participants in the VACIRiSS Trial**



*Other exclusion criteria met include: Core temperature ≥38.0°C within the past 24 hours (n=13); Hypersensitivity to PCV13 or diphtheria toxoid-containing vaccines (n=3); Recent vaccination (n=12); Pregnant and lactating (n=52); Nursing home/long-term care facility/other / requires nursing care (n=83); Platelet count less than 50 x 109/L and/or INR greater than 1.3 (n=96); Splenectomy (n=11)

**Other reasons for exclusion include: Discharged prior to eligibility check (n=180); Geography - Patient lives too far away for follow-up (n=134); Non-English speaker (n=29); Mental capacity (n=65); Imminent transfer to non-participating hospital (n=60); Enrolled in other studies without co-enrolment agreement with VACIRiSS trial (n=99); Research staff unavailable (n=137); Clinician decision (n=70); Recruitment on hold (n=39); Noncompliant with care/ social history (n=53); Other (n=23); Unknown (n=53).

14. Diagnosis and main criteria for inclusion

Eligible participants were adults (≥18 years of age) admitted to ICU with sepsis, whose clinical condition had improved and were ready for general ward concentration care.

Patients who met all the following inclusion criteria and none of the exclusion criteria were eligible to participate in the trial.

14.1 Inclusion Criteria

i. Male or female adult patients aged 18 years or older on the date of screening for the trial
ii. Registered with a General practitioner
iii. Reason for admission to intensive care unit or high dependence unit was sepsis
iv. Clinical condition has improved, and the patient is ready for step down to high dependency unit (HDU) or ward-based care in the next 24-48 hours
v. Provision of written informed consent by the patient OR by patient's Legal representative OR professional consultee.

14.2 Exclusion Criteria

Patients who met one or more of the following were excluded from the trial.

i. Core temperature ≥38.0°C within the past 24 hours prior to study IMP administration. As with other vaccines, the administration of Prevenar 13 should be postponed in subjects suffering from acute, **severe** febrile illness. However, the presence of a **minor** infection, such as a cold, should not result in the deferral of vaccination.
ii. Hypersensitivity reaction (e.g., anaphylaxis) to any component of Prevenar 13 or any diphtheria toxoid-containing vaccine.
iii. Recent vaccination defined as any vaccination administered to subjects within 7 days of enrolment.
iv. Pregnant and lactating women.
v. Limitations of care set including not for resuscitation, not for readmission to critical care
vi. Residence in a nursing home, long-term care facility, or other institution, or requirement of semiskilled nursing care. An ambulatory subject who was a resident of a retirement home or village is eligible for the trial. The rationale for this exclusion criteria being an explanatory trial with multiple follow-up, which was considered onerous for this patient group.
vii. As the IMP is administered intra muscularly, coagulopathy defined as platelet count less than 50 x 10^9/L and/or INR greater than 1.3. For this exclusion criteria bloods taken within 72 hours of screening visit are valid. If these standard of care blood results are not available, then these should form part of the screening bloods for assessing eligibility.
viii. Splenectomy (previous or in the current admission)

ix. Diagnosis of pneumococcal sepsis in the current admission

x. APACHE II score defined Immune deficiency or suppression, defined as presence of 1 or more of the following conditions:
   - Documented human immunodeficiency virus (HIV) infection at any time-point pre-trial. If previous results are not available and/or current admission is not due to HIV infection, these patients do not need new testing and are considered eligible for the trial.
   - Leukaemia (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
   - Lymphoma (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
   - Hodgkin disease (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
   - Multiple myeloma (presence defined as having been treated by or been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
   - Malignancy (defined as presence of any malignancy that had been treated by or had been eligible for treatment by radiotherapy and/or chemotherapy within the last 5 years)
   - Chronic renal failure (defined as receipt of renal dialysis or transplant) or nephrotic syndrome
   - Receipt of immunosuppressive therapy, including steroids, within 3 months of study vaccine administration (For corticosteroids, prednisone or equivalent 0.5 mg/kg/day for 14 days or longer. Inhaled, intra- articular, and topical steroids are not considered immunosuppressive.
   - Receipt of an organ or bone marrow transplant with ongoing immunosuppressive medications. Failed previous transplant patients not currently on immunosuppression are eligible.

Contraception: In the context of post critical illness hospitalisation it was assumed that patients would be sexually abstinent, which is considered a highly effective method of contraception. A pregnancy test was permitted at screening or at any time during the current hospital admission to exclude pregnant women. Patients were hospitalised at the time of screening and baseline until 7-10 days following the IMP administration. Therefore, females of child bearing age were eligible for the trial without need for any additional testing or contraception.

15. Test product, reference therapy, dose and mode of administration

Investigational Medicinal Product

15.1 Active

Prevenar 13® is a Pneumococcal Polysaccharide conjugate vaccine (13 valent, adsorbed) suspension for injection marketed by Pfizer Limited. The vaccine is a homogenous white suspension.

The IMP was supplied by Pfizer and distributed to individual participating sites via Guy's and St Thomas's Hospital NHS Foundation Trust pharmacy.

Each trial site had a delegated pharmacy team and/or research nurses, who were un-blinded to the intervention and prepared the blinded product using aseptic technique for administration. The product was administered by the blinded research team.

The Prevenar 13® solution was drawn up into a syringe in accordance with an aseptic worksheet and labelled with an Annex 13 label. The label was attached around the barrel of the syringe to mask the contents.

15.2 Placebo

The placebo used in this study was sodium chloride 0.9% w/v solution for injection. This was supplied locally by each participating site; any UK licensed product was allowed for this study. A volume of 0.5ml was drawn up into a syringe and labelled with an Annex 13 label. The label was attached around the barrel of the syringe to mask the contents.

15.3 Dosing Regimen

Participants were randomised 1:1 to receive one single 0.5ml dose of active or placebo study IMP at baseline. IMP was administered within 72 hours of randomisation (if the participant deteriorated in that time, IMP administration was delayed until clinically well). The dose was administered by intramuscular (IM) injection.

15.4 Duration of treatment

A single dose of IMP was administered at Baseline (T0) following randomisation.

16. Criteria for evaluation: Endpoints

16.1 Primary endpoint

Differences in time to first infection related rehospitalisation or death within the 365 days follow-up period between the intervention and control arm.

16.2 Secondary endpoints

Generating precision estimates for the proportion of rehospitalisation, proportions of reinfections, proportion of reinfection related rehospitalisation, and time to first antibiotic therapy in general practice at different follow-up time points within the 365 days follow-up period between the intervention and control arm.

16.3 Exploratory endpoints of vaccine effects and immune recovery patterns

Vaccine specific effect was assessed using anti-pneumococcal antibody. The differences between the interventional and control arms will inform vaccine effect and the control arm changes will inform how the immune system recovers in sepsis survivors.

- Differences in anti-pneumococcal antibody at baseline and 30 (+/-7) days between the intervention and control arm will be assessed
- Heterologous vaccine effects and immune recovery patterns: Immune recovery patterns and heterologous vaccine effects will be measured at baseline (T0) before IMP administration, on 10 (+/-3) days post T0 in patients still in hospital, on 30 (+/-7) days' post T0 and 90(+/-7) days post T0. This will include flow cytometry (B cell subsets, T cell subsets and monocyte HLA-DR and PD-1 expression), function and leukocyte transcriptome differences between the intervention and control arm.
- Define vaccine responder characteristic

16.4 Safety endpoints

The safety endpoints assessed in trial participants were local reactions (pain, redness, and swelling at the study vaccine injection site and limitation of arm movement), systemic reactions (fever, diarrhoea, chills, fatigue, headache, vomiting, decreased appetite, rash, new generalized muscle pain, new generalized joint pain). Table 4 summarises safety monitoring. Local and systemic reactions were monitored from IMP administration to day 7. All other AEs were monitored up to 30 days. After the 30-day period, the monitoring was limited to infections, and SAEs only.

Table 4: Safety evaluation of trial participants

Safety data	D1 - vaccination	Follow-up visits days post-IMP administration				
		10 (+/-3 days)	30 (+/-3 days)	90 (+/-7 days)	180 (+/-7 days)	365 (+/-14) days
Local adverse reactions	X	X				
Systematic adverse reactions	X	X				
Serious adverse events including Rehospitalisation**	X	X	X	X	X	X
Adverse events including infections	X	X	X	X*	X*	X*

* Refers to infections only

** Planned hospital admissions are exempt from SAE reporting

17. Statistical Methods

Statistical Analysis of Clinical Outcomes

17.1 Sample size

Our systematic reviews (5, 6), highlighted an expected rate for infection-related hospitalization or death by 365 days of 32.1%. No prior information was available on the expected treatment effect for PCV13 in sepsis survivors. A sample size of 214 would provide 90% power to detect a hazard ratio (HR) of 0.36 for the primary outcome compared with PCV13 with placebo ($P<0.05$).

17.2 Clinical outcomes

Participants were analyzed according to the group assigned at randomization. For the primary outcome, Kaplan-Meier survival curves and incidence rate for each group are reported and compared between groups using incidence rate ratio (IRR) and difference (IRD) calculated within age strata (≤65 vs. >65 years) and combined using internally standardized weights proportional to person-days of follow-up in the intervention group. Analyses were repeated for the two components of the composite primary outcome to assess consistency of the direction of treatment effect. As death is a competing event for time to first infection-related rehospitalization, death was treated as censoring, corresponding to a "while-on-treatment" strategy (63).

For the binary secondary outcomes, we report the number and percentage of participants experiencing the event in each group at pre-specified follow-up timepoints, with the risk ratio (RR) between groups using a generalized linear model with a log link, adjusted for age and SSIP score (linear) and with robust standard errors clustered by site. For time-to-event secondary outcomes, we report HR using cause-specific Cox proportional hazards models, adjusted for age and SSIP score (linear) and shared frailty (random effect) at the site level, with deaths treated as censoring, corresponding to a "while-on-treatment" strategy (*63*). The analytic details of HRQoL are reported below.

17.3 Safety

Pre-defined local reactions (pain, swelling, limitations of arm movement) and systemic events (fever, muscle and joint pain, headache) between T0 and T1 were reported using the vaccine diary. Severe adverse events (SAEs) including relatedness to the intervention were recorded.

17.4 Sensitivity analysis

To account for any potential impact of the COVID-19 pandemic, for the primary outcome, we report the HR (95% CI) for PCV13 compared with placebo with a Cox proportional hazards model restricted to participants randomized up to February 1, 2020, and with outcomes censored on February 29, 2020. The impact of the COVID-19 pandemic on the trial recruitment is reported in section 8.2. There was no imputation of missing clinical data. Analyses were unadjusted for multiplicity. All statistical tests were two-sided with significance set at $P<0.05$, and effect estimates reported with 95% confidence intervals (95% CI). Clinical outcomes analyses were performed in Stata/MP version 18.0.

17.5 Predictors in the Sepsis Survivor Prognosis (SSIP) score

Predictors associated with all rehospitalizations or death in the first year after surviving hospitalization for sepsis-related critical illness included in the parsimonious prognostic score. The final SSIP score had an area under the receiver operating characteristic curve of 0.673 (95% CI, 0.670 to 0.677).

The score included 4 categories based on the risk of outcome events. Risk stratum=1 (low; 0 – 4 points); Risk stratum=2 (5 – 6 points); Risk stratum=3 (7 – 10 points) and Risk stratum = 4 (highest; ≥11 points).

Table 5: Predictors in the Sepsis Survivor Prognosis (SSIP) score

Risk factor known at index sepsis admission	Points	Risk factor known at index sepsis admission	Points
(a) **Number of hospitalizations in previous year**		**(e) Co-morbidities**	
- 0	0	- 0	0
- 1	1	- 1	2
- 2	2	- 2	3
- 3 or more	4	- 3	4
		- 4+	5
(b) Age in 20-year increments		**(f) Admission**	
- <40	0	- Elective surgical	0
- 40 – 59	1	- Emergency surgical	1
- 60 – 79	2	- Medical	2
- 80+	3		
(c) Deprivation quintile		**(g) Hemoglobin at admission**	
- 1-4	0	- >9	0
- 5 (most deprived)	1	- 7.1 – 9	1
		- ≤7	2
(d) Pre-admission dependence		**(h) Site of infection**	
- None	0	- Neurologic	0
- Moderate	2	- Respiratory	1
- All	5	- Cardiovascular	2
		- Gastrointestinal	1
		- Genitourinary	1
		- Musculoskeletal/Dermatologic	0
		- Unknown	1

17.6 Additional methods for health-related quality of life (HRQoL) analyses

The mean and standard deviation of the 'your health today' visual analog scale was reported in each arm at baseline and at each follow-up timepoint but not modelled or tested. The percentage of patients reporting each level of each domain of the EQ-5D-5L at each follow-up timepoint in each arm was displayed in stacked bar charts.

For exploratory clinical outcome of HRQoL we report the mean (SD) of the EQ-5D-5L index score[2] in each group at baseline and at each follow-up timepoint and compared between groups using a linear mixed model, adjusted for baseline EQ-5D-5L, age and SSIP score (linear) and random effects at the site level.

18. Changes in the Trial Plan

A pre-specified statistical analysis plan was written and approved before the start of statistical analysis. There were no changes to the planned analysis.

18.1 Protocol Deviations

No serious breaches or any major protocol deviations occurred and therefore no impact on the analysis.

19. Summary – Conclusions

19.1: Table 6. Baseline characteristics of Study Participants in the VACIRiSS Trial

Description of trial population at randomization

Characteristic	PCV13 (N = 104)	Placebo (N = 110)
Age (years), mean (SD)	60.5 (15.0)	59.9 (14.2)
Age strata, No,/total No. (%) [a]		
≤65 years	58/104 (55.6)	62/110 (56.4)
>65 years	46/104 (44.4)	48/110 (43.6)
Sex, No./total No. (%)[a]		
Female	48/104 (46.2)	43/110 (39.1)
Male	56/104 (53.8)	67/100 (60.9)
Self-reported ethnicity, No./total No. (%)[a]		
White	98/104 (94.2)	97/110 (88.2)
Asian	0/104 (0.0)	3/110 (2.7)
Black	4/104 (3.8)	7/110 (6.4)
Mixed	1/104 (1.0)	2/110 (1.8)
Other	1/104 (1.0)	1/110 (0.9)
Charlson comorbidity index, No./total No. (%)[a]		
0	41/103 (39.8)	44/110 (40.0)
1	27/103 (26.2)	33/110 (30.0)
2	15/103 (14.6)	17/110 (15.5)

3	10/103 (9.7)	9/110 (8.2)
4+	10/103 (9.7)	7/110 (6.4)
PCV vaccination any time prior, No./total No. (%)[a]		
Yes	24/104 (23.3)	19/110 (17.3)
No	64/104 (62.1)	78/110 (70.9)
Unknown	16/104 (15.5)	13/110 (11.8)
Influenza vaccination last 12 months, No./total No. (%)[a]		
Yes	39/104 (37.9)	39/110 (35.5)
No	59/104 (57.3)	59/110 (53.6)
Unknown	6/104 (5.8)	12/110 (10.9)
Surgical status, No./total No. (%)[a]		
Elective surgery	17/104 (16.3)	10/110 (9.1)
Emergency surgery	24/104 (23.1)	23/110 (20.9)
Medical	63/104 (60.6)	77/110 (70.0)
Pre-admission dependence, No./total No. (%)[a]		
None	93/104 (89.4)	85/110 (77.3)
Moderate (some assistance with ADLs)	10/104 (9.6)	25/110 (22.7)
Severe (total assistance with ADLs)	1/104 (1.0)	0/110 (0.0)
Pre-admission residence, No./total No. (%)[a]		
Home	100/104 (96.2)	108/110 (98.2)
Health-related institution	3/104 (2.9)	0/110 (0.0)
Non-health related institution	0/104 (0.0)	2/110 (1.8)
No fixed address/abode or temporary abode	1/104 (1.0)	0/110 (0.0)
Primary site of infection, No./total No. (%)[a]		
Respiratory	38/103 (36.9)	46/110 (41.8)
Gastrointestinal	31/103 (30.1)	24/110 (21.8)
Genitourinary	15/103 (14.6)	14/110 (12.7)
Musculoskeletal	7/103 (6.8)	4/110 (3.6)
Bloodstream infection (including endocarditis and catheter-related infection)	4/103 (3.9)	12/110 (10.9)
Other[b]	4/103 (3.9)	9/110 (8.2)
Unknown	4/103 (3.9)	1/110 (0.9)
SSIP score[c], No./total No. (%)[a]		
0-4	20/104 (19.2)	19/110 (17.3)
5-6	29/104 (27.9)	30/110 (27.3)
7-10	40/104 (38.5)	45/110 (40.9)
11+	15/104 (14.4)	16/110 (14.5)
White blood cell count (x10^9 cells/L), median (IQR)	11.0 (8.1, 13.6) [N = 102]	9.9 (7.0, 12.7) [N = 107]

C-reactive protein (mg/L), median (IQR)	64 (20, 121) [N = 101]	54 (21, 123) [N = 101]
APACHE II score[d], mean (SD)	16.5 (6.4) [N = 103]	16.8 (7.9) [N = 108]
SOFA score[e], median (IQR)	7 (4, 9) [N = 103]	7 (4.5, 10) [N = 108]

a Percentages may not sum to 100 because of rounding.

b other sites of infection were dermatological (PCV13, 4; placebo, 4); neurological (PCV13, none; placebo, 4) and surgical wound (PCV13, none; placebo, 1).

c The SSIP score ranges from 0 to 22 points, with four pre-defined categories where lower scores indicate decreased risk of outcome: 0-4 points (low-risk); 5-6 points; 7-10 points and ≥11 points (highest risk). The score consists of eight independent predictors measured at the index sepsis admission, namely: the number of hospitalizations in the year preceding the index sepsis admission, age in 10-year increments, residence in a postcode within the highest quintile of deprivation, preadmission dependence (defined and categorized as none, moderate (some assistance required with activities of daily living (ADLs) or severe (total assistance required with ADLs), number of comorbidities, admission type, blood hemoglobin at index sepsis admission, and site of infection.

d The Acute Physiology And Chronic Health Evaluation (APACHE) II score is a severity of illness score that ranges from 0 to 71 with increased scores corresponding to a greater risk of death.

e The Sequential Organ Failure Assessment (SOFA) score assesses organ dysfunction in six body systems, assigning a score from 0 (normal) to 4 (failure) for each organ, with the total score ranging from 0 to 24.

Abbreviations: PCV13, 13-valent conjugate pneumococcal vaccine; SD, standard deviation; IQR, interquartile range

19.2 Primary clinical outcome

By 365 days after randomization, there were first events (3 deaths and 40 infection-related rehospitalizations) among 72.5 person-years of follow-up for 103 participants in the PCV13 group (0.59 per person-year, 95% CI, 0.43 to 0.80) compared with 38 first events (2 deaths and 36 infection-related rehospitalizations) among 76.5 person-years of follow-up for 109 participants in the placebo group (0.50 per person-year, 95% CI, 0.35 to 0.68), corresponding to an incidence-rate ratio (IRR) of 1.17 (95%CI, 0.75 to 1.81), IRD of 0.09 (95%CI, -0.15 to +0.33) and hazard ratio (HR) of 1.23 (95%CI, 0.80 to 1.91; p=0.35) with a Cox proportional hazards model. The IRR (95% CI) stratified by age for the primary outcome was 1.51 (0.78 to 2.95) in participants ≤65 years compared with 0.91 (0.46 to 1.79) in participants >65 years (test for heterogeneity, p=0.26) (Fig.3A; Fig.4A).

As the primary outcome is a composite, the IRR (95% CI) for the two components of the primary outcome, time to first infection-related rehospitalization and time to death, were 1.15 (0.73 to 1.80) and 1.18 (0.40 to 3.52), respectively. There were 7 deaths among 97.9 person -

years of follow-up for 103 participants in the PCV13 group compared with 6 deaths among 101.1 person-years of follow-up for 109 participants in the placebo group (Fig.3A).

We had previously reported the SSIP score (Section 17.5) (31), that predicts unplanned all-cause rehospitalization or death in the first year after hospital discharge for adult sepsis survivors. We assessed heterogeneous treatment effects on the primary outcome with a Cox proportional hazards model, adjusted for the stratification variable of age and SSIP score (linear) and shared frailty (random effect) at the site level. The treatment effects for increasing SSIP score risk categories were similar (test for heterogeneity, p=0.55) (Table 8).

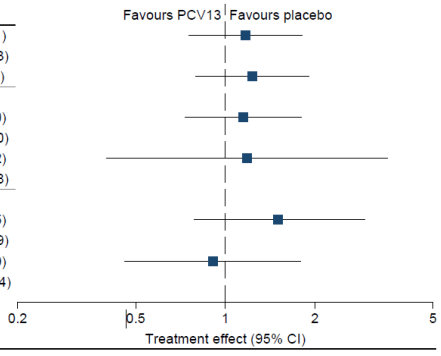
19.3 Secondary clinical outcomes

The proportions of all-cause rehospitalization and infection-related rehospitalizations at 30, 90, 180, and 365-days were broadly similar between groups (Fig.3B-3C). These observations were consistent with the HR (95% CI) of 1.12 (0.77 to 1.61) for time to first all-cause hospitalization. The proportions(%) (n/N) of reinfection events at 30, 180 and 365-days in the PCV13 group versus placebo groups were 44.1% (45/102) vs 32.7% (34/104); 58.4% (59/101) vs 50.0% (52/104), and 69.3% (70/101) vs 59.6% (62/101), respectively. The corresponding risk-ratio (RR) (95% CI) at 30, 180 and 365-days were 1.31 (1.05 to 1.63), 1.15 (1.01 to 1.31) and 1.22 (1.11 to 1.35), respectively (Fig.3D). The HR (95% CI) for time to first antibiotic therapy in general practice was of 1.34 (0.95 to 1.88) (Fig.4B). The mean (SD) health-related quality of life (HRQoL) at baseline in the PCV13 and placebo groups were similar, with no differences in EQ-5D utility scores at subsequent time points (Table 9; Fig.6).

19.4: Figure 3: Primary and secondary outcomes

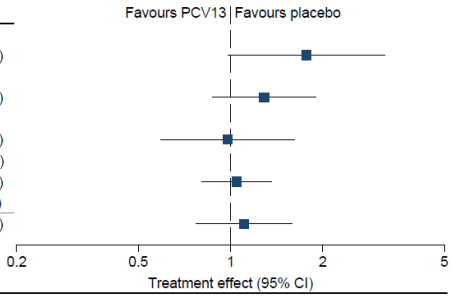
A

Outcome	No. / person-yrs at risk (rate) PCV13	Placebo	Treatment effect (95% CI)
Time to first infection-related rehospitalisation or death (primary outcome)	43/72.5 (0.59)	38/76.5 (0.50)	IRR 1.17 (0.75, 1.81)
			IRD 0.09 (-0.15, 0.33)
			HR 1.23 (0.80, 1.91)
Components of primary outcome			
Time to first infection-related rehospitalisation	40/72.5 (0.55)	36/76.5 (0.47)	IRR 1.15 (0.73, 1.80)
			IRD 0.07 (-0.16, 0.30)
Time to death	7/97.9 (0.07)	6/101.1 (0.06)	IRR 1.18 (0.40, 3.52)
			IRD 0.01 (-0.06, 0.08)
Primary outcome by age strata			
≤65 years	24/40.9 (0.59)	18/46.3 (0.39)	IRR 1.51 (0.78, 2.95)
			IRD 0.20 (-0.10, 0.49)
>65 years	19/31.6 (0.60)	20/30.2 (0.66)	IRR 0.91 (0.46, 1.79)
			IRD -0.06 (-0.46, 0.34)



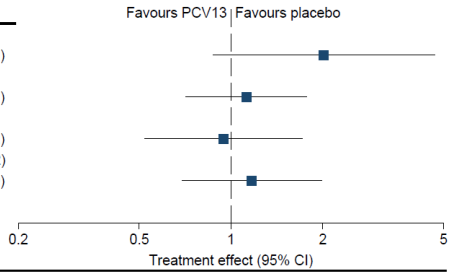
B

Outcome	No. / total No. or person-yrs at risk (% or rate) PCV13	Placebo	Treatment effect (95% CI)
All-cause rehospitalisation			
By 30 days	19/102 (18.6)	11/104 (10.6)	RR 1.77 (0.98, 3.19)
			RD 8.1 (-1.5, 17.6)
By 90 days	40/101 (39.6)	32/104 (30.8)	RR 1.29 (0.87, 1.91)
			RD 8.8 (-4.2, 21.9)
By 180 days	43/101 (42.6)	45/104 (43.3)	RR 0.98 (0.59, 1.62)
			RD -0.7 (-14.2, 12.9)
By 365 days	59/101 (58.4)	58/104 (55.8)	RR 1.05 (0.81, 1.36)
			RD 2.6 (-10.9, 16.2)
Time to first all-cause rehospitalisation	59/57.8 (1.02)	58/61.7 (0.94)	HR 1.11 (0.77, 1.59)



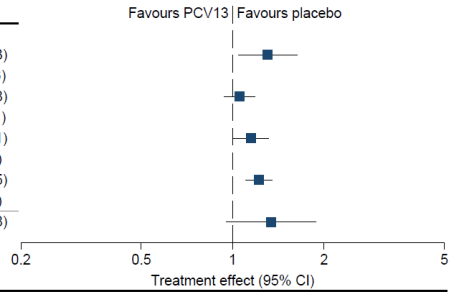
C

Outcome	No. / total No. (%) PCV13	Placebo	Treatment effect (95% CI)
Infection-related rehospitalisation			
By 30 days	12/102 (11.8)	6/104 (5.8)	RR 2.02 (0.87, 4.67)
			RD 6.0 (-1.7, 13.7)
By 90 days	25/101 (24.8)	23/104 (22.1)	RR 1.12 (0.71, 1.77)
			RD 2.6 (-9.0, 14.2)
By 180 days	28/101 (27.7)	30/104 (28.8)	RR 0.95 (0.52, 1.72)
			RD -1.1 (-13.5, 11.2)
By 365 days	40/101 (39.6)	36/104 (34.6)	RR 1.17 (0.69, 1.99)
			RD 5.0 (-8.2, 18.2)



D

Outcome	No. / total No. or person-yrs at risk (% or rate) PCV13	Placebo	Treatment effect (95% CI)
Reinfection			
By 30 days	45/102 (44.1)	34/104 (32.7)	RR 1.31 (1.05, 1.63)
			RD 11.4 (-1.8, 24.6)
By 90 days	52/101 (51.5)	50/104 (48.1)	RR 1.05 (0.94, 1.18)
			RD 3.4 (-10.3, 17.1)
By 180 days	59/101 (58.4)	52/104 (50.0)	RR 1.15 (1.01, 1.31)
			RD 8.4 (-5.2, 22.0)
By 365 days	70/101 (69.3)	62/104 (59.6)	RR 1.22 (1.11, 1.35)
			RD 9.7 (-3.3, 22.7)
Time to first antibiotic therapy in general practice	71/45.0 (1.58)	63/54.0 (1.17)	HR 1.34 (0.95, 1.88)



Death refers to all-cause mortality. Reinfections include Infection-related rehospitalizations and infections treated in primary care. All cause rehospitalizations include Infection-related rehospitalizations. *Rate is reported per person-year of follow-up for all time to event outcomes: the primary outcome of time to first infection related rehospitalization or death from any cause within 365-days, the components of the primary outcome, and the secondary outcomes of time to first all-cause rehospitalization and time to first antibiotic therapy in general practice. The hazard ratios reported in relative effect were

derived from the corresponding Cox regression models.

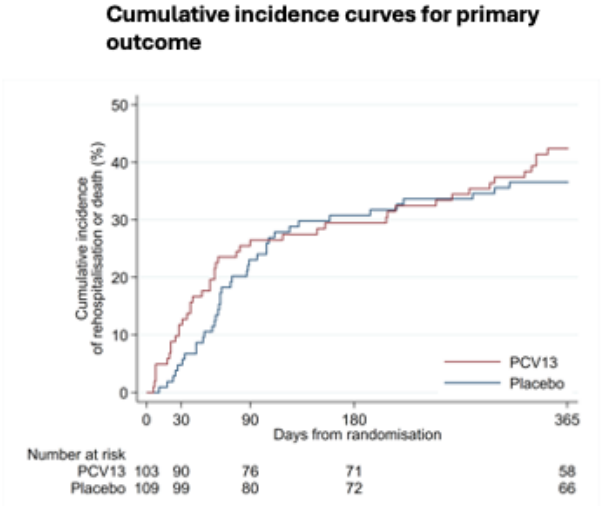
Fig.3A: Primary outcome overall, components of primary outcome, and primary outcome by age strata
Fig.3B: Secondary outcome of all-cause rehospitalization as binary variable at pre-specified follow-up time points shown include 30 days, 90 days, 180 days and 365 days, and time to first all-cause rehospitalization during the 365-day follow-up period.
Fig.3C: Secondary outcome of infection-related rehospitalization as binary variable at prespecified follow-up time points shown include 30 days, 90 days, 180 days and 365 days
Fig.3D: Secondary outcome of reinfection events as binary variable at pre-specified follow-up time points shown include 30 days, 90 days, 180 days and 365 days, and time to antibiotic therapy in general practice during the 365-day follow-up period.
Abbreviations: PCV13, 13-valent conjugate pneumococcal vaccine; IRR, incidence rate ratio; HR, hazard ratio; CI, confidence interval; IRD, incidence rate difference; RR, relative risk; RD, risk difference.

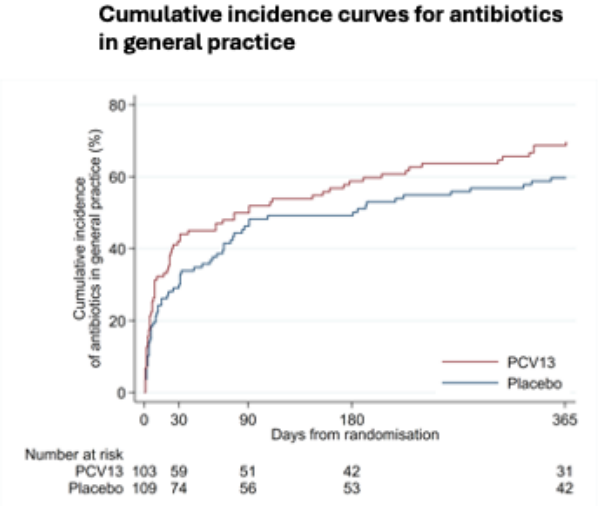
19.5: Figure 4: Cumulative incidence plots

Fig.4A: Cumulative incidence with 95% CI of infection-related rehospitalization or all-cause mortality up to 365-days following randomization.

Fig.4B: Cumulative incidence with 95% CI of time to antibiotics in general practice up to 365-days following randomization.

Additional pre-specified follow-up time points shown include 30 days, 90 days, 180 days and 365 days. PCV13, 13-valent conjugate pneumococcal vaccine.





19.6 Safety outcomes

The occurrence of prespecified local reactions and systemic events were similar between the PCV13 and placebo groups (Fig.5). There were 125 SAEs among 54 of 104 participants (51.9%) in the PCV13 group and 75 SAEs among 39 of 110 participants (35.4%) in the placebo group (p=0.02; SAE by MedDRA System Organ Class is reported in Table 7). None of the SAEs were reported as vaccine related.

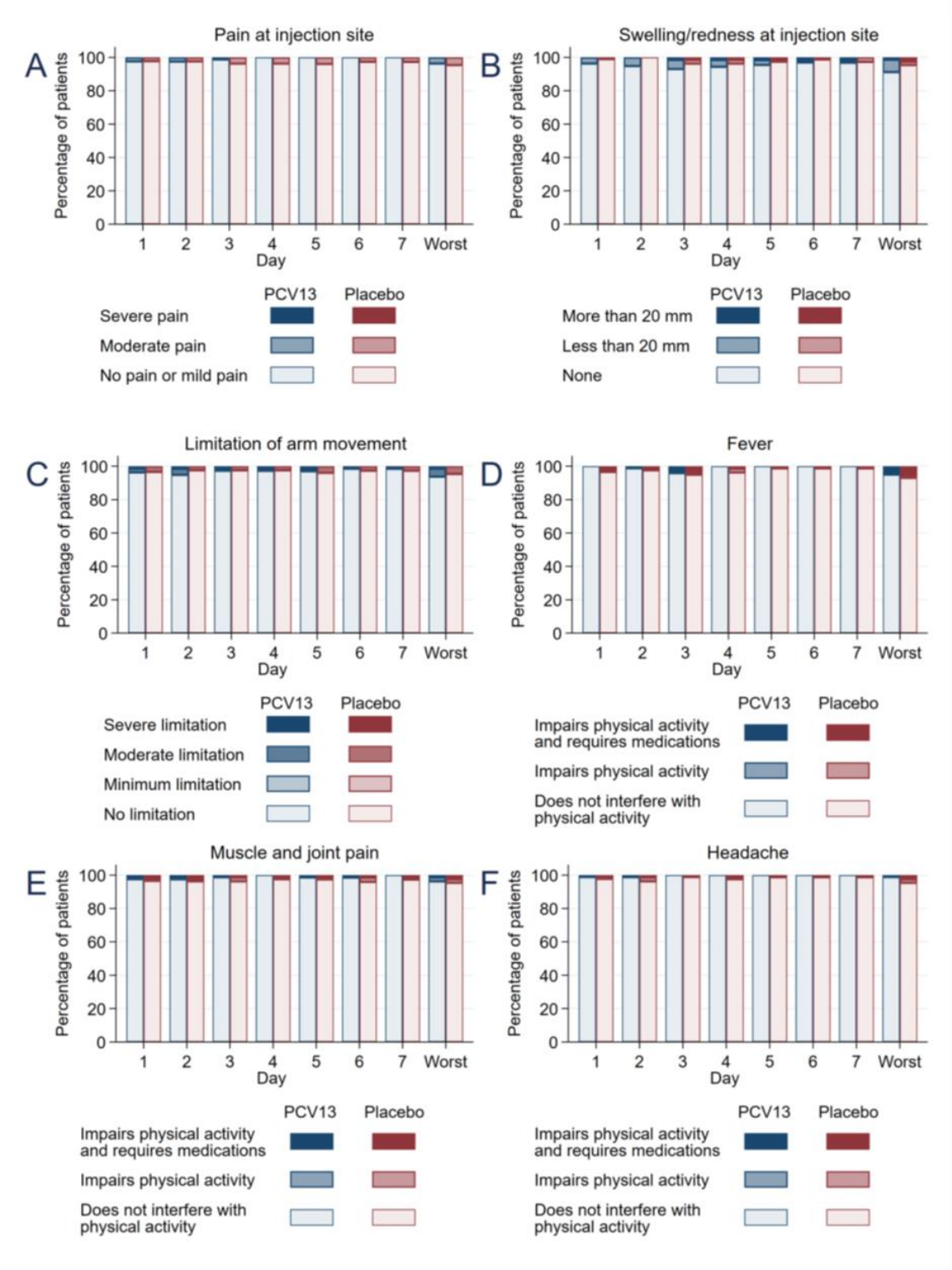
Table 7: Serious adverse event summary by MedDRA System Organ Class

System Organ Class	PCV13		Placebo	
	No. of participants (%)	No. of events	No. of participants (%)	No. of events
Cardiac disorders	5 (4.81)	6	4 (3.64)	7
Gastrointestinal disorders	12 (11.54)	19	5 (4.55)	5
General disorders and administration site conditions	7 (6.73)	8	2 (1.82)	2
Hepatobiliary disorders	1 (0.96)	2	2 (1.82)	2
Infections and infestations	25 (24.04)	35	26 (23.64)	37
Injury, poisoning and procedural complications	8 (7.69)	8	3 (2.73)	3
Investigations	1 (0.96)	1	0 (0.00)	0
Metabolism and nutrition disorders	6 (5.77)	16	1 (0.91)	1
Musculoskeletal and connective tissue disorders	1 (0.96)	1	1 (0.91)	1
Neoplasms benign, malignant and unspecified	0 (0.00)	0	1 (0.91)	1
Nervous system disorders	3 (2.88)	3	2 (1.82)	3
Renal and urinary disorders	2 (1.92)	3	1 (0.91)	1
Respiratory, thoracic and mediastinal disorders	9 (8.65)	9	4 (3.64)	6
Skin and subcutaneous tissue disorders	3 (2.88)	4	1 (0.91)	1
Surgical and medical procedures	6 (5.77)	7	4 (3.64)	4
Vascular disorders	3 (2.88)	3	2 (1.82)	3
Any	54 (51.92)	125	39 (35.45)	77

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; PCV13, 13-valent conjugate pneumococcal vaccine.

Figure 5: Safety events (local and systemic) recorded in the vaccine diary

We report the grades of severity in pre-defined local reactions (pain, swelling, limitations of arm movement) and systemic events (fever, muscle and joint pain, headache) between T0 and T1 were reported in the vaccine diary.



19.7 Sensitivity analyses

For the primary outcome, to account for the COVID-19 pandemic, we restricted to participants randomized up to 1 February 2020 and with outcomes censored on 29 February 2020 and note that the treatment effects were consistent with the main analysis (HR 1.11 95%CI, 0.58 to 2.11; p=0.75; Table 8).

Table 8: SSIP score subgroups and overall sensitivity analysis

Analysis	PCV13		Placebo		Hazard ratio (95% CI)	P value
	Total No.	No./person-yrs (rate)	Total No.	No./person-yrs (rate)		
Subgroup analysis by SSIP category						
Risk stratum 1 (low, 0-4 points)	19	3/16.3 (0.18)	19	4/15.3 (0.26)	0.71 (0.16, 3.16)	0.55[a]
Risk stratum 2 (5-6 points)	29	11/21.0 (0.52)	29	6/24.6 (0.24)	2.19 (0.81, 5.97)	
Risk stratum 3 (7-10 points)	40	19/25.3 (0.75)	45	19/28.0 (0.68)	1.16 (0.61, 2.20)	
Risk stratum 4 (highest, ≥11 points)	15	10/9.6 (1.04)	16	9/8.3 (1.09)	0.96 (0.39, 2.40)	
Sensitivity analysis - pre-COVID-19	48	20/18.5 (1.08)	47	18/16.0 (1.13)	1.11 (0.58, 2.11)	0.75

Abbreviations: CI, confidence interval; PCV13, 13-valent conjugate pneumococcal vaccine; SSIP, Sepsis Survivor Prognosis score.
a P value for test of interaction

19.8 Quality of Life Outcomes

Figure 6: HRQoL measured with EQ-5D-5L

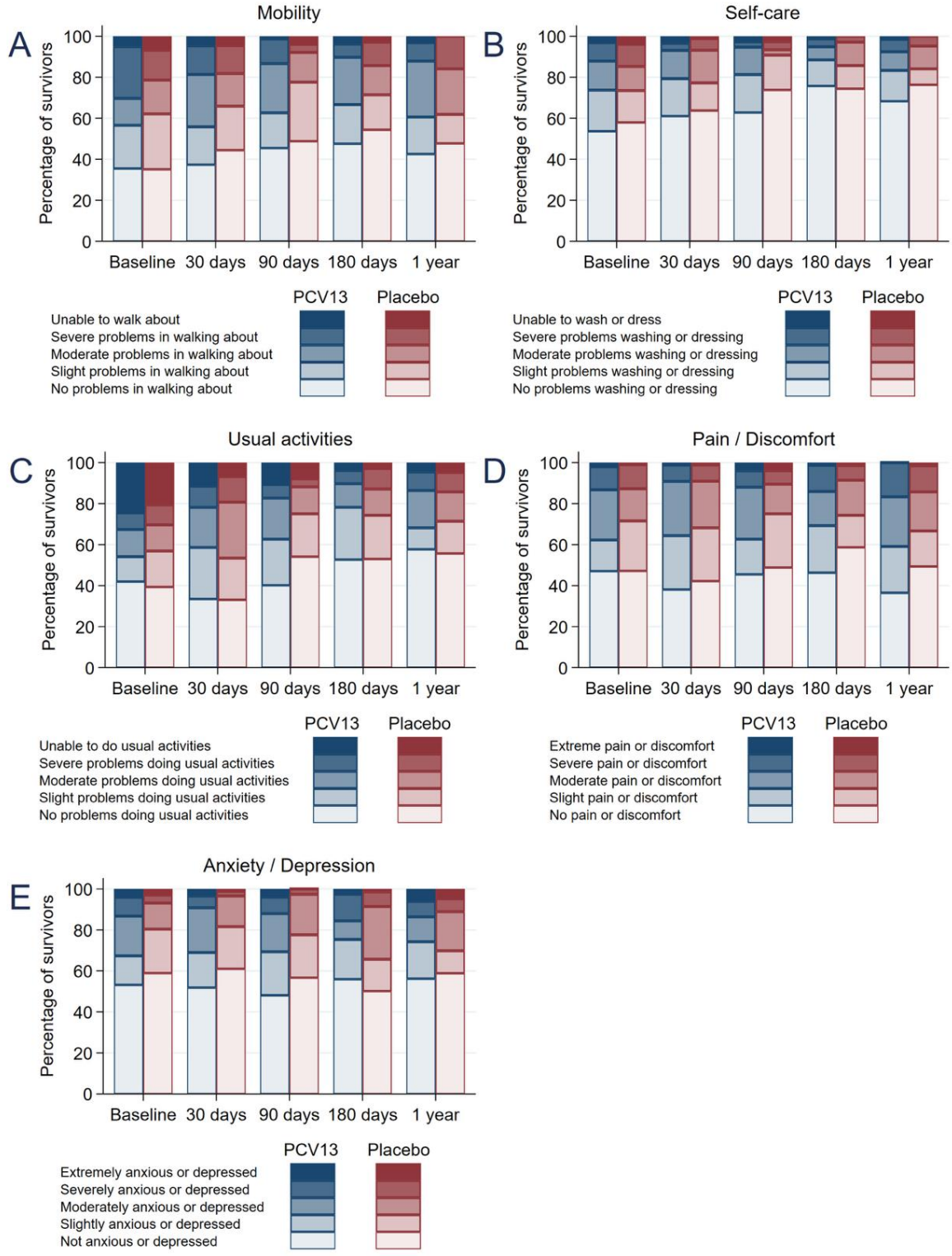


Table 9: Health Related Quality of Life results

Outcome	Mean (SD) [No. of participants]		Mean difference (95% CI)	P value
	PCV13	Placebo		
EQ-5D utility				
Baseline	0.57 (0.32) [97]	0.61 (0.33) [102]		
30 days	0.61 (0.30) [87]	0.66 (0.29) [87]	-0.04 (-0.11, 0.04)	0.30
90 days	0.61 (0.34) [78]	0.70 (0.29) [77]	-0.04 (-0.13, 0.05)	0.41
180 days	0.65 (0.33) [81]	0.67 (0.33) [74]	-0.01 (-0.10, 0.08)	0.82
365 days	0.59 (0.34) [73]	0.62 (0.37) [69]	-0.00 (-0.11, 0.10)	0.98
EQ-5D visual analog scale				
Baseline	50.3 (27.6) [97]	60.5 (25.0) [101]		
30 days	62.3 (23.7) [87]	64.6 (23.2) [88]		
90 days	65.9 (24.2) [76]	74.8 (17.8) [75]		
180 days	64.9 (26.1) [78]	70.2 (24.6) [71]		
365 days	66.2 (26.1) [65]	69.2 (25.3) [62]		

Abbreviations: CI, confidence interval; PCV13, 13-valent conjugate pneumococcal vaccine; EQ-5D, EuroQol 5-dimension.

19.9 Biological outcomes

All biological outcomes of vaccine immunogenicity were defined a priori as exploratory outcomes for the trial and measured blinded to treatment allocation and clinical outcomes. For biological outcomes with longitudinal measurements, we compared the corresponding time points between the randomized groups as is convention in trials. We also report within group longitudinal changes to explore PCV13 immunogenicity effects, as described in previous vaccine response studies (29, 30, 32, 33).

19.9.1 PCV13 specific serology

We assessed serotype-specific immunoglobulin-G (IgG), to the 13 vaccine serotypes of Streptococcus pneumoniae (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F), at T0 and T2 to coincide with peak IgG responses to vaccination(34), at the World Health Organization (WHO) reference Laboratory for Pneumococcal Serology based at the Institute of Child Health, University College London (35).

At T0, serotype-specific IgG concentrations were similar between the PCV13 and placebo groups (Fig.7A). At T2, IgG concentrations for the 4, 7F, 9V, 18C, 19A, and 23F serotypes were significantly increased in the PCV13 group compared with the placebo group (Fig.7B-7F; Fig.A1; Table 10). We observed wide variations in serotype-specific IgG responses, with the highest fold change from T0 to T2 observed for serotypes 4, 23F, 6A, 18C and 14 (Fig.7G). The correlations between different serotype specific IgG were modest (Fig.7H).

Figure 7: PCV13 serotype specific antibodies

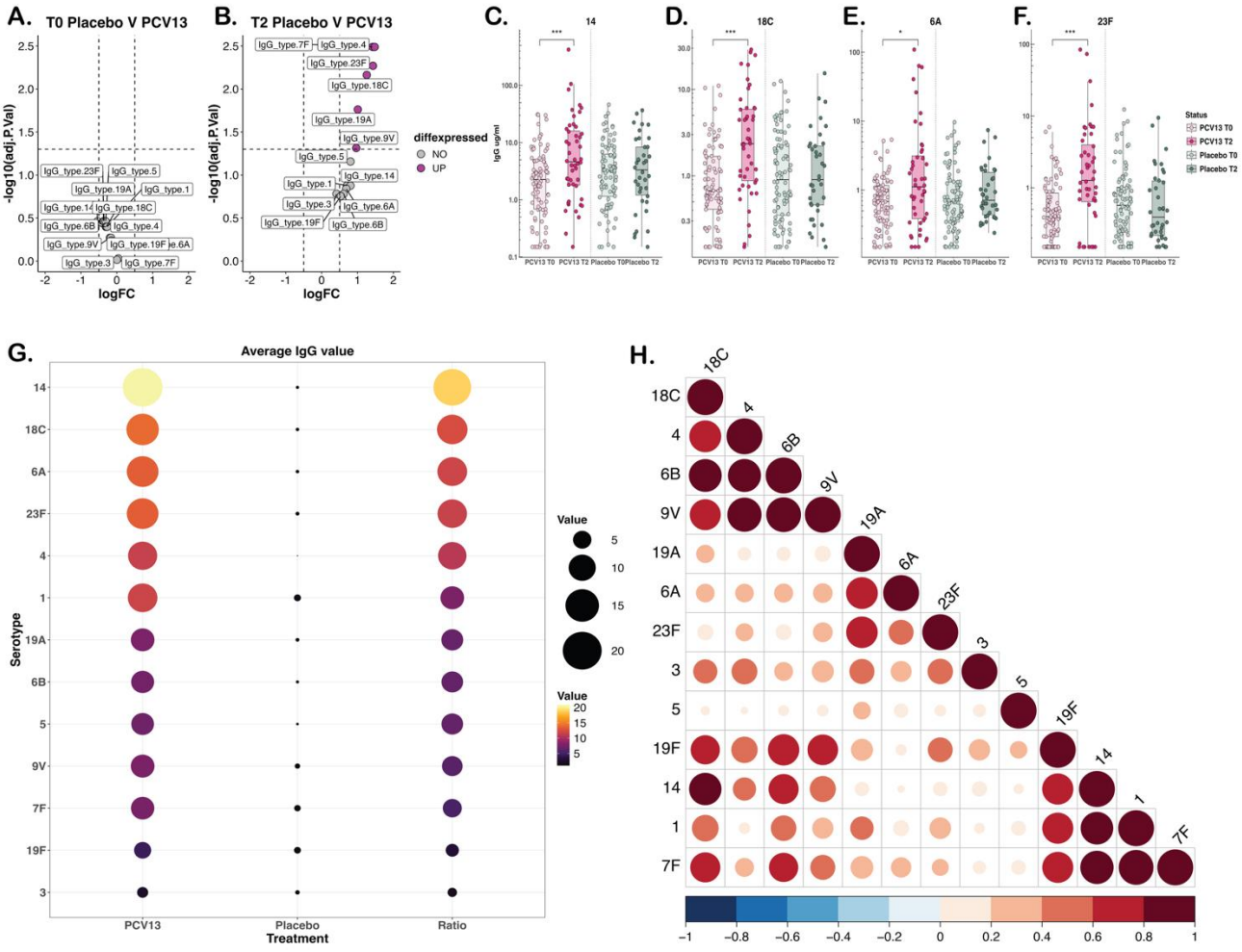


Table 10: Serotype specific anti-pneumococcal immunoglobulin G (IgG) in the PCV13 group and in the placebo groups

Serotype specific IgG levels (pg/ml)	PCV13		Placebo	
	Baseline (T0)	30(±7) days (T2)	Baseline (T0)	30(±7) days (T2)
1	0.4 (0.2 – 0.8); (n=82)	1.1 (0.4 – 3.6); (n=44)	0.5 (0.2 – 1.1); (n=89)	0.7 (0.3 – 1.6); (n=37)
3	0.2 (0.2 – 0.2); (n=63)	0.2 (0.2 – 0.6); (n=35)	0.2 (0.2 – 0.2); (n=66)	0.2 (0.2 – 0.2); (n=26)
4	0.3 (0.2 – 0.6); (n=90)	1.1 (0.3 – 2.7); (n=48)	0.3 (0.2 – 0.6); (n=93)	0.4 (0.2 – 0.6); (n=38)
5	0.5 (0.3 – 0.8); (n=88)	1.1 (0.7 – 3.4); (n=46)	0.6 (0.3 – 1.2); (n=92)	0.9 (0.3 – 1.6); (n=38)

6A	0.7 (0.4 – 1.2); (n=90)	1.1 (0.4 – 3.1); (n=48)	0.7 (0.3 – 1.3); (n=93)	0.7 (0.4 – 1.8); (n=38)
6B	0.5 (0.3 – 0.9); (n=90)	0.9 (0.4 – 3.9); (n=45)	0.6 (0.3 – 1.4); (n=91)	0.7 (0.4 – 1.4); (n=34)
7F	0.7 (0.4 – 1.65); (n=90)	1.82 (0.8 – 5.6); (n=48)	0.6 (0.3 – 1.9); (n=91)	0.8 (0.3 – 1.8); (n=38)
9V	0.7 (0.3 – 1.5); (n=91)	1.7 (0.8 – 6.5); (n=46)	0.7 (0.4 – 1.6); (n=91)	0.8 (0.5 – 1.6); (n=38)
14	2.6 (0.9 – 4.8); (n=86)	4.8 (1.8 – 16.4); (n=47)	3.2 (1.4 – 6.5); (n=91)	3.7 (1.4 – 8.4); (n=37)
18C	0.7 (0.4 – 1.7); (n=90)	2.4 (0.9 – 5.9); (n=48)	0.9 (0.3 – 2.5); (n=92)	0.9 (0.4 – 2.2); (n=36)
19A	1.8 (1 – 3.1); (n=91)	6.3 (2.9 – 9.8); (n=48)	2.2 (1.3 – 4.8); (n=93)	3.7 (1.7 – 5.6); (n=37)
19F	1.3 (0.6 – 2.4); (n=90)	3.3 (1.6 – 5.53); (n=45)	1.5 (0.7 – 2.7); (n=91)	1.6 (0.9 – 2.8); (n=38)
23F	0.4 (0.2 – 0.8); (n=91)	1.3 (0.6 – 4); (n=48)	0.6 (0.2 –1); (n=93)	0.4 (0.2 – 1.2); (n=38)

Abbreviations: PCV13, 13-valent conjugate pneumococcal vaccine.
Values are median (interquartile range). n represents the number of samples with results.

19.9.2 Lymphocyte subsets

Sepsis commonly affects adults ≥60 years (2), where age-associated changes in immune system, including in lymphocyte subsets are prevalent (36, 37) and could influence PCV13 immunogenicity. Further, in healthy adults ≥60 years, previous study indicates weaker PCV13 responses associated with reduced T_H1 cells (30). Thus, we hypothesized that sepsis survivors may have variable PCV13 immunogenicity, as sepsis is associated with increased T_H2 (38, 39) and regulatory T (Tregs) cells (40, 41). We assessed B and T cell subsets in the peripheral blood mononuclear cell (PBMC) fraction, informed by the Human Immunology Project guidance (HIP guidance) on standardized immunophenotyping using flowcytometry (42), with the memory B cell subset gating additionally informed by Berkowska and colleagues (43) (Fig.A2-A3). For comparisons, we recruited age-matched healthy controls with a concurrently running cohort study (44).

At T0, T1 and T2, the proportions of CD4 cells and CD4 subsets were largely similar between the PCV13 and placebo groups (Fig.A4A-A4Q). However, compared with age-matched healthy controls, participants in both groups had significantly decreased proportions of CD4+ naïve (Fig.A4C), increased proportions of CD4 central memory (Fig.A4E) and decreased proportions of CD4 effector memory (Fig.A4F). The CD4 effector memory increased over time only in the PCV13 group (Fig.A4F). The T_H1 subset significantly increased over time, with a corresponding decrease in the T_H2 subsets only in the PCV13 group (Fig.A4H). The proportions of Th17 (Fig.A4I) and Tregs, including subsets (Fig.A4J-A4L) decreased over time in both PCV13 and placebo groups, compared with age-matched healthy controls. As hypothesized, at T0, T1 and T2 both groups had increased proportions of T_H2

than T_H1 indicating that the T_H2 skewing in sepsis (38, 39) persists in sepsis survivors, and could partly explain the observed variation in serotype-specific PCV13 immunogenicity (30, 45).

At T0, T1 and T2, the proportions of CD8 cells and CD8 subsets were largely similar between the PCV13 and placebo groups, with a greater variation in the PCV13 group (Fig.S7MS7Q).
Compared with age-matched healthy controls, participants in the PCV13 and the placebo groups had significantly decreased proportions of CD8+ naïve (Fig.S7N), increased proportions of CD8 effector and effector memory subset (Fig.S7O-S7P), decreased proportions of CD8 central and effector memory (Fig.S7Q).

At T0, T1 and T2, the proportions of B cells and B cell subsets were similar between the PCV13 and placebo groups (Fig.A5A-A5H). However, compared with age-matched healthy controls, participants in both groups had significantly decreased proportions of B cells (17.4% vs 11.7% vs 11.3% in healthy controls, PCV13 and placebo respectively; (Fig. A5A), decreased proportions of transitional B cells (Fig. A5B) and increased proportions of plasmablasts (Fig.A5G). In contrast with the placebo group, the naive mature B cell subsets were significantly increased at T2 (44.4%) compared with T0 (35.7%) and T1 (32.4%), in the PCV13 group (Fig.A5C). Amongst the memory B cell subsets, the natural effector subsets (Fig.A5D), IgM memory (Fig.A5E), and IgG memory (Fig.A5F) were largely similar between the PCV13 and placebo groups, with IgG memory subsets marginally increased in PCV13 group compared with age-matched healthy controls (Fig.A5F).

Table 11: Proportions of T helper cells and subsets

Variable Median (IQR) proportions	Healthy	PCV13			Placebo		
		Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)	Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)
CD3 (% of live)	59 (56.3 - 61.2); (33)	63 (46.7 – 69.9); (44)	65.8 (61.4 – 73.4); (34)	66.8 (59.7 -73.4); (29)	56.2 (44.7 - 73.2); (40)	62.8 (51.2 -72.7); (34)	61.2 (56.2 – 69.6); (25)
CD4	66 (65.6 - 66.3); (33)	66.6 (48.4 –76.7); (44)	65 (51.4 – 78.1); (34)	65 (47.1 - 74.5); (29)	67 (59.3 - 77.3); (40)	67.2 (56.1 – 74.8); (34)	62.4 (53.3 –76.1); (25)
CD4 Central Memory	23.1 (19.8 - 26.6); (33)	42.6 (29.2 – 63.8); (44)	41.6 (30.1 – 57.4); (34)	38.5 (18.5 -29.8); (29)	37 (26.8 - 66.7); (40)	38.7 (26.8 – 66.7); (34)	37.8 (24.3 – 54.4); (25)

CD4 Effector	1.55 (1.25 – 2.1); (33)	0.75 (0.29 – 3.01); (44)	1.12 (0.34 – 2.92); (34)	0.8 (0.58 - 3.44); (29)	1.14 (0.24 – 2.5); (40)	1.19 (0.46 – 2.22); (34)	1.05 (0.44 – 2.21); (25)
CD4 Effector Memory	27.1 (24.7 – 27.9); (33)	16.2 (10.9 – 24.8); (44)	17.2 (10.5 – 33.6); (34)	25.5 (15.6 - 38.4); (29)	17.6 (10.7 – 24.6); (40)	16.6 (10.7 – 23.2); (34)	16.7 (11.3 – 28.3); (25)
CD4 Naive	48.8 (45.4 – 51.2); (33)	27.8 (14 – 41.7); (44)	25 (12.5 – 46.2); (34)	22.8 (16.1 - 36.1); (29)	29.6 (10.8 – 49.4); (40)	32.4 (10.1 – 50.7); (34)	33.3 (9.76 – 55); (25)
T_H1	10.5 (9.36 – 10.9); (33)	11.2 (6.55 – 19.9); (44)	13.3 (8.32 – 23.8); (34)	14.2 (11.4 - 26); (29)	10.6 (6.3 – 19.8); (40)	13.8 (8.71 – 21.4); (34)	18.1 (6.8 – 21.9); (25)
T_H2	68.9 (67.1 – 74.2); (33)	71.8 (62.4 – 83.9); (44)	63.6 (54.4 – 78.9); (34)	66.3 (56.5 - 78); (29)	76.9 (60.9 – 83.4); (40)	71.6 (58.5 – 77.9); (34)	71.1 (62.9 – 80.7); (25)
T_H17	13.5 (11.2 – 15.4); (33)	7.68 (4.98 – 14.6); (44)	9.02 (6.97 – 13.1); (34)	8.41 (4.98 - 15.1); (29)	8.71 (5.09 – 11.3); (40)	8.35 (3.92 – 12.3); (34)	6.46 (2.6 – 10.2); (25)
T_{Regs}	8.02 (7.35 – 9.46); (33)	6.76 (5.94 – 9.8); (44)	6 (4.69 – 7.83); (34)	6.51 (3.78 - 8.59); (29)	6.56 (4.63 – 8.7); (40)	6.26 (4.63 – 8.7); (34)	6.69 (5.01 – 7.37); (25)
Memory T_{Regs}	14.7 (3.66 – 42.9); (33)	44.5 (10.7 – 65.4); (44)	32.2 (9.51 – 64); (34)	35.5 (7.75 - 53.2); (29)	46 (10.1 – 66.8); (40)	35.5 (7.75 – 53.2); (34)	32.2 (4.72 – 57.4); (25)
Naive T_{Regs}	36.7 (9.82 – 47.7); (33)	18.6 (9.56 – 47.8); (44)	16.4 (8.99 – 35.3); (34)	20.1 (5.4 - 40.2); (29)	13 (4.76 – 27.8); (40)	13.8 (5.36 – 24.1); (34)	7.7 (2.1 – 21.3); (25)
CD8	29.7 (29.5 – 30.1); (33)	25.2 (17.2 – 42.4); (44)	24.8 (15.6 – 43.2); (34)	29.2 (19.2 -46.6); (29)	23.8 (16.9 – 31.4); (40)	24.9 (18 – 32.6); (34)	26.1 (14.5 – 35); (25)

CD8 Central Memory	9.86 (8.1 – 11.9); (33)	13.9 (9.67 – 11.9); (44)	14.2 (9.02 – 18.4); (34)	12.9 (10.1 - 16.9); (29)	14.6 (7.88 – 26.8); (40)	17 (6.63 – 25.5); (34)	16 (6.8 – 24.4); (25)
CD8 Effector	6.87 (5.88 – 10.2); (33)	31.6 (18.3 – 53.2); (44)	32.9 (15.8 – 54.2); (34)	39.7 (18.2 - 53.5); (29)	30.6 (17.2 – 40.5); (40)	30.3 (19 – 41.1); (34)	27 (15.7 – 40.6); (25)
CD8 Effector Memory	44.2 (42.4 – 49.2); (33)	18.6 (10.1 – 34.5); (44)	20.4 (12.6 – 37.9); (34)	25.9 (13.1 – 34.6); (29)	23.2 (14.8 – 44.8); (40)	30.2 (16.7 – 46.4); (34)	28.5 (15.8 – 41.9); (25)
CD8 Naive	37.1 (36 – 38.4); (33)	13.2 (6.27 – 37.1); (44)	15.5 (6.1 – 39.4); (34)	12.5 (5.37 – 32.9); (29)	12.4 (4.66 – 30.1); (40)	15 (3.97 – 25.6); (34)	12.7 (6.04 – 34.4); (25)

Table 12: Proportions of B cell subsets

Variable Median (IQR) proportions	Healthy Median (IQR); (n)	PCV13			Placebo		
		Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)	Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)
CD19 (%of live)	17 (15.3 - 19.8); (24)	9.66 (5.23 – 18.1); (30)	8.58 (5.02 – 12.29); (24)	8.22 (6.1 – 10.4); (17)	8.42 (5.03 -14); (29)	7.98 (4.57 – 11.42); (26)	8.44 (3.67 – 13.85); (18)
B1	0.65 (0.65 – 0.52); (24)	0.75 (0.39 – 2.66); (30)	1.66 (0.877 – 2.28); (24)	1.41 (0.51 – 2.26); (17)	0.86 (0.32 - 2); (29)	1.44 (0.54 – 4.12); (26)	0.7 (0.46 – 2.26); (18)
Natural Effector	7.63 (4.44 – 11.4); (24)	8.14 (4.71 – 10.75); (30)	8.19 (5.02 – 10.42); (24)	6.24 (3.33 – 11.5); (17)	5.38 (3.33 - 8.65); (29)	6.52 (3.64 – 8.75); (26)	6.45 (3.69 – 9.74); (18)
Unswitched memory	2.47 (1.91 – 3.21); (24)	1.76 (1.12 – 2.57); (30)	1.94 (1.34 –2.8); (24)	1.9 (1.29 – 2.91); (17)	2.77 (1.4 - 4.51); (29)	2.54 (1.23 – 5.02); (26)	2.68 (1.32 – 3.15); (18)
CD27- IgG+	1.64 (1.52 – 2.04); (24)	1.86 (1.16 – 4.12); (30)	1.7 (1.07 – 4.16); (24)	2.17 (0.77 – 3.31); (17)	2.67 (0.99 - 4.45); (29)	2.12 (0.7 – 5.32); (26)	2.73 (1.03 – 5.22); (18)
IgG Memory	3.38 (2.82 – 3.66); (24)	4.37 (1.74 – 6.49); (30)	4.77 (2.36 –8.59); (24)	2.23 (0.37 – 6.65); (17)	3.02 (0.53 – 7.33); (29)	3.90 (0.17 – 8.28); (26)	3.92 (0.24 – 8.66); (18)

Naïve Mature	41.1 (38.4 – 45.6); (24)	35.3 (23.5 – 45); (30)	31 (20.2 – 41.8); (24)	45.2 (37.4 – 52.7); (17)	32.5 (23.6 – 50.2); (29)	42.2 (30 – 50.5); (26)	38.6 (31.6 –43.2); (18)
Plasmablasts	0.23 (0.11– 0.32); (24)	1.04 (0.39 – 3.58); (30)	2.17 (0.53 – 4.16); (24)	0.42 (0.08 –1.66); (17)	3.46 (1.38 – 10.7); (29)	1.52 (0.23 – 5.24); (26)	1.08 (0.38 – 4.71); (18)
Transitional	2.46 (1.79– 3.07); (24)	0.63 (0.28 – 0.97); (30)	0.56 (0.29 –0.88); (24)	0.29 (0.08 –0.74); (17)	0.64 (0.26 –1.39); (29)	0.5 (0.2 – 0.8); (26)	0.42 (0.19 – 0.86); (18)

Abbreviations: IQR, interquartile range; PCV13, 13-valent conjugate pneumococcal vaccine.
Values are median (interquartile range) of proportions. The B cell subsets reported include Naïve, Unswitched memory, Class Switched memory and plasmablasts, as a proportion of CD19+ cells. These longitudinal measurements were at T0, T1 (i.e., 10 (+/-3) days post T0) and at T2

19.9.3 Plasma cytokines

Our selection of measured plasma cytokines were hypotheses driven. The expected cytokine abnormalities in sepsis survivors include abnormal interleukin-6 (IL-6), IL-8, IL-17A and IL-10 concentrations (10). The B cell survival factors (B cell-activating factor (BAFF), and a proliferation-inducing ligand (APRIL) that contribute to survival of plasma cells induced by immunization (46) and CD40L involved in helper T cell signals needed for germinal center formation and immunoglobulin isotype switching are acutely altered in sepsis (17, 47, 48).
Vaccine immunogenicity varies with interleukin-17 (IL-17), IL-22, and IL-6 concentrations of at the time of vaccine administration (22), with IL-6 and IL-17A also associated with heterologous vaccine effects (22) and CXCL10, a plasma cell differentiation factor (49). For comparisons, we recruited age-matched healthy controls with a concurrently running cohort study (44).

At T0, T1 and T2, the cytokine concentrations were similar between the PCV13 and placebo groups (Fig.A5I-A5P). Compared with age-matched controls, the PCV13 and placebo groups had increased concentrations of BAFF, APRIL, sCD40L, and IL-8, at T0, T1 and T2, and increased concentrations of IL-6 and IL-10 at T0 and T1. Both PCV13 and placebo groups had variable concentrations of CXCL10. These abnormalities suggest ongoing innate and adaptive immune responses in sepsis survivors, alongside inflammation and immunosuppression.

Table 13: Cytokine concentrations in the PCV13 group and in the placebo groups

Variable	Healthy	PCV13			Placebo		
		Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)	Baseline (T0) Median (IQR); (n)	10+/- 3 days (T1) Median (IQR); (n)	30+/- 7days (T2) Median (IQR); (n)
BAFF	5.06 (0 – 48); (27)	302 (145 – 606); (96)	265.2 (128 – 523.5); (73)	296.2 (180 – 665.8); (51)	330 (141.7 – 903.5); (102)	416.7 (194.2 – 658); (72)	320.7 (200.6 –

							711.2); (40)
APRIL	298 (225 – 476); (27)	2545.3 (1220.2 – 4853.5); (96)	2757.5 (1489.1 – 5057.6); (73)	3049.2 (1780.3 – 5479.7); (51)	3064 (1439 – 5865); (102)	3801 (1482 – 5324); (72)	2949 (1735 – 4223); (40)
CD40L	28.2 (13.5 – 84.6); (27)	142 (59.7 – 757.1); (96)	156 (51.8 – 732.7); (73)	193.4 (59.6 – 469.6); (51)	166.9 (57.1 – 893.8); (102)	133.6 (60.6 – 779.2); (72)	126.1(53.7 – 379.8); (40)
IL-6	2.51 (1.14 – 4.66); (27)	11 (4.8 – 23.9); (96)	7 (3 – 16.2); (73)	3.3 (1– 8.2); (51)	12.5 (3.5– 24.4); (102)	3.9 (1.9– 12.4); (72)	3.3 (1.5 – 5.6); (40)
IL-10	0.86 (0.65 – 1.33); (27)	1.3 (0.9 – 2.4); (96)	1.2 (0.8 – 1.6); (73)	1.1 (0.8 – 1.5); (51)	1.3 (0.8 – 2.4); (102)	1.4 (0.9 – 2.2); (72)	0.9 (0.6 – 1.6); (40)
IL-17A	0.64 (0.54 – 0.95); (27)	0.8 (0.5 – 1.6); (96)	0.7 (0.5 – 0.9); (73)	0.5 (0.4 – 0.9); (51)	0.7 (0.6 – 1.2); (102)	0.7 (0.5 – 1); (72)	0.6 (0.4 – 1.1); (40)
CXCL-8	0.04 (0.0 – 0.55); (27)	6.2 (3.5 – 10.4); (96)	5.2 (2.8 – 9); (73)	4 (2.4 – 7.3); (51)	5.7 (3 – 17); (102)	4.4 (2.9 – 10.6); (72)	3.7 (2 – 8.2); (40)
CXCL-10	95.4 (68.7 – 109); (27)	107.6 (44.6 – 185.2); (96)	96.5 (58.3 – 148.2); (73)	104.3 (60.3 – 152.9); (51)	118.4 (52.7 – 206.7); (102)	98.2 (59.1 – 118.8); (72)	88.4 (67.9 – 118.7); (40)
IFNy	0.92 (0.01 – 4.58); (27)	2.42 (1.31 – 4.92); (96)	2.48 (0.91 – 4.61); (73)	3.1 (1.28 – 5.44); (51)	2.4 (1.67 – 6.93); (102)	2.55 (1.45 – 4.33); (72)	2.70 (1.87 – 4.5); (40)

Abbreviations: PCV13, 13-valent conjugate pneumococcal vaccine. Values are median (interquartile range). The cytokines reported include B cell-activating factor (BAFF), a proliferation-inducing ligand (APRIL), soluble cluster of differentiation 40 ligand (CD40L), interleukins (IL-6, IL-10, IL-17A) and chemokines (CXCL8, CXCL10).

19.9.4 Differentially expressed genes and Blood transcriptional modules (BTMs) in the panleukocyte transcriptome

We assessed the panleukocyte transcriptome in PCV13 and placebo groups at T0, T1 and T2. We mapped 59,735 genes out of the 61,544 genes listed in GENCODE GRCh38, filtered lowly expressed genes (n=33,568) and analyzed 26,167 genes with Deseq2 (50). Using the likelihood ratio test (LRT), between the PCV13 and placebo groups, we identified 64 differentially expressed genes (DEG) at T0, 21 DEG at T1 and 13 DEG at T2 (Fig.A6A-A6C). Our explanation for the minimal DEG between the between the PCV13 and placebo groups at T0, T1 and T2 is that signals from PCV13 effects on the panleukocyte transcriptome were masked by the ongoing transcriptional responses in sepsis

survivors.

This specific challenge with DEG analyses in vaccine transcriptome studies has been previously addressed using BTMs (29, 32), Briefly, panleukocyte transcriptome data were curated into large-scale interaction networks of co-regulated genes (33, 51) that drive cellular function/mechanisms (52, 53). Thus, we compared the similarities and differences in the myeloid, B and T cell BTMs at T1 vs T0, and at T2 vs T0, within the PCV13 and placebo groups.

Among the myeloid BTMs, the NK cell associated modules (such as M7.2, M61, S1) had increased expression, in the T1 vs T0 and in T2 vs T0 comparisons, in both the PCV13 and placebo groups. In contrast, the monocyte associated modules of antigen presentation (M200, M95, M71) and myeloid, dendritic cell activation via NFkB module (M43.0) were decreased only in the placebo group, in the T2 vs T0 comparisons (Fig.8A). Among the B cell BTMs (Fig.8B), naïve B cell associated signatures (S2, S8) and naïve and memory B cell module (M83) had increased expression in the T1 vs T0 and in T2 vs T0 comparisons, only in the placebo group. The BCR signaling module (M54) had increased expression in both the PCV13 and placebo groups in the T1 vs T0 comparisons, decreased only in the placebo group by T2 vs T0 comparisons. In addition, plasma cell associated module (M156.1) and the signature (S3) also decreased only in the placebo group by T2 vs T0 comparisons. Among the T cell BTMs (Fig.8C), at T1 vs T0 comparisons, the T cell activation (M52) and the IL2/IL7/TCR network (M65) modules had increased expression only in the PCV13 group. Among the T cell modules at T2 vs T0 comparisons, the key differences were the enrichment of IL2/IL7/TCR network (M65) and T cell activation (M52) modules in the PCV13 groups.

These findings indicate that there are ongoing immune responses in sepsis survivors, and that responses varied between them. We also observed that several innate and lymphocyte related responses were modified by PCV13, along with evidence of T cell dependent effects from PCV13 in sepsis survivors.

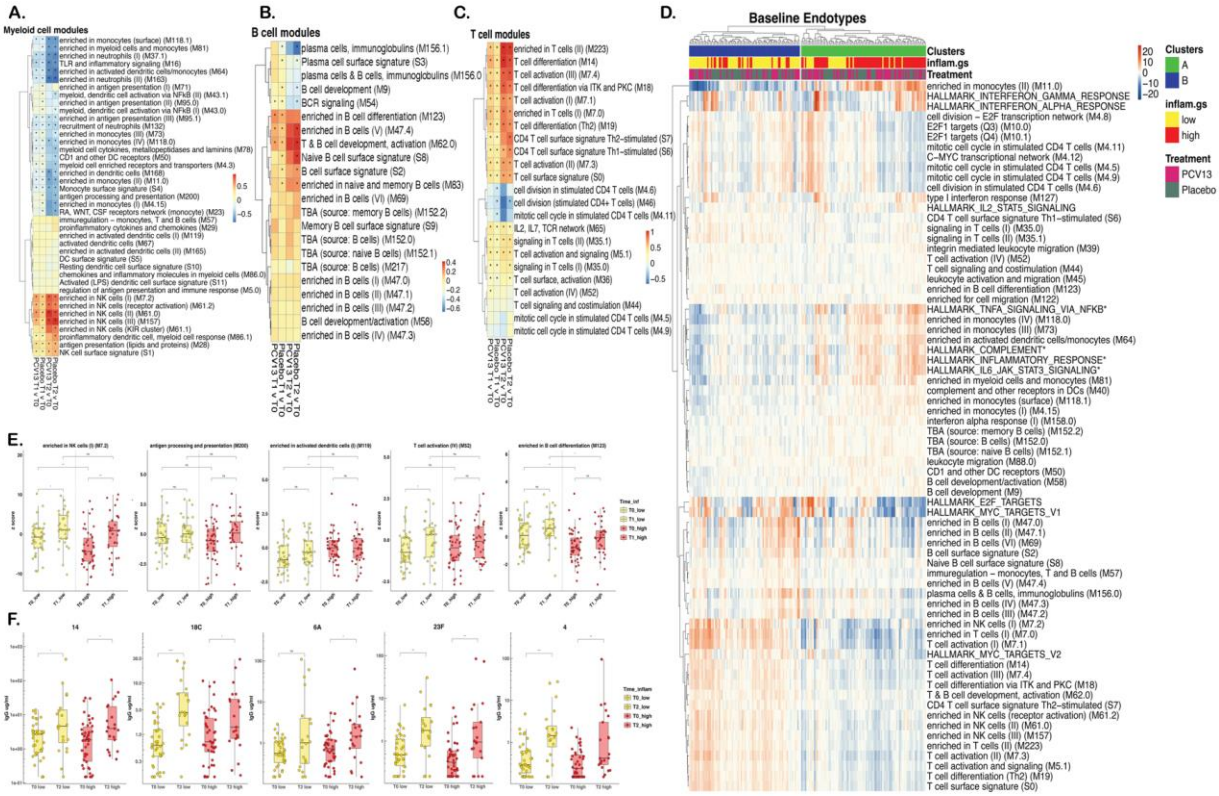
19.9.5 Pre-vaccination innate immune endotypes (29)

The above variation in transcriptomic responses led us to explore mechanistic subsets within the trial population. Pre-vaccination innate immune states in health have been grouped into three endotypes based on BTMs associated with pro-inflammatory response, cell proliferation, and metabolism alterations based on the four Hallmark gene sets - Inflammatory response, Complement, IL6 JAK STAT3 signaling and TNFA signaling via NFKB (29). Greater antibody responses were associated with high inflammation endotype enriched on the innate immune activation state triggered by Toll-like receptor ligands or adjuvants (29). Our trial population had evidence of innate immune inflammation and PCV13 is an adjuvant vaccine. Thus, we hypothesized and assessed whether similar innate immune endotypes identified using sample-level enrichment analysis (SLEA)(29) based on the same four Hallmark gene sets and could explain the variation in serotype-specific IgG and enrich responders to PCV13 in the trial population.

Our data indicated a two-cluster model (Clusters A and B) as best fit for our data (Fig.8D). We

categorized our trial population with the median values of the four hallmark inflammatory gene sets used by Fourati and colleagues (29) to derive the high and low pre-vaccination innate immune inflammatory endotypes. In cluster-A, the prevalence of high and low inflammation endotypes were (n/N=70/97 (72.2%)), and (n/N=27/97 (27.8%)) respectively. In cluster-B, the prevalence of high and low inflammation endotypes were (n/M= 22/86 (25,6%)), and (n/N=64/86 (74.4%)) respectively. On average, the increase in the serotype-specific IgG between T0 and T2 was similar between the high and low inflammation endotypes, with variations in the magnitude of change between serotypes between endotypes (Fig.8e and Fig.A6D). Thus, although these endotypes partly explain the observed variations in serotype-specific IgG, they are unlikely to enrich responders to PCV13 in a sepsis survivor population.

Figure 8: Blood Transcriptome Modules (BTM) and pre-vaccination innate immune endotypes



Heatmaps of mean activity scores of blood transcriptomic modules from Qusage comparisons at T1 vs T0 and T2 vs T0 in PCV13 and in Placebo groups are shown Fig.4A: Myeloid cells blood transcriptomic modules; Fig.4B: B cell blood transcriptomic modules; Fig.4C: T cell blood transcriptomic modules; Fig.4D: Hierarchical clustering of pre-vaccination T0 samples based on the expression of the BTMs and hallmark gene sets using sample-level enrichment analysis (SLEA) highlights two clusters and two innate immune inflammatory endotypes inflammation-high and inflammation-low. *Refers to the four hallmark inflammatory gene sets used to determine endotypes; Fig.4E: Box and Whisker plot showing examples of blood transcriptomic modules by inflammation-high and inflammation-low endotypes; Fig.4F: Box and Whisker plot showing serotype-specific IgG by inflammation-high and inflammation-low endotypes; * = p<0.05; **=p<0.01; ***p<0.001 using non-parametric tests (False discovery rate 0.05 for modules).

19.9.6 Clinical and immunological parameters associated with PCV13 antibody responses

The endotype analyses reported above led us to consider additional clinical and immunological features associated with and explain the variations in serotype-specific IgG responses in participants randomized to PCV13 group within our trial. These covariates were informed by a recent study that involved in nineteen healthy adults ≥60 years of age, that highlighted several clinical (age, sex, body mass index (BMI)) and immunological (TH subsets, immunoglobulin heavy chain genes (IGHG, IGHA, IGHM), cytotoxicity-associated gene module (CYTOX module), plasmablasts activity score) features associated with PCV13 immune responses using univariate correlation analyses (30).

We observed a greater decline in serotype specific IgG responses with age in men and with increased body mass index (BMI) in women (Fig.9A-9B), which are consistent with results from healthy adults ≥60 years (30). In contrast with the observations in healthy adults ≥60 years (30), we observed no statistically significant differences in serotype-specific IgG response between male and female, despite the average age of 60.5 (15.0) years, and 55% of trial participants were ≥65 years. Unlike the observations in healthy adults (30), we did not observe significant correlations between the serotype-specific IgG responses and the T helper subsets (T_H1, T_H2, T_H17) or their corresponding ratios (T_H2/ T_H1, T_H1/ T_H17) (Fig 9C). Although there were no differences in the immunoglobulin heavy chain genes (IGHM, IGHG1, IGHG2, IGHG3, IGHA1, IGHA2) between the PCV13 and placebo groups at baseline (Fig 9D), the immunoglobulin heavy chain genes were lower in the high inflammation endotype at T0, when compared with low inflammation endotype within the PCV13 group (Fig 9E) and there was no statistically significant increase in these genes by T1 in either endotypes (Fig 9F). Although there were no differences in the genes within the cytotoxicity-associated genes (CYTOX) between the PCV13 and placebo groups at baseline (Fig 9G), the CYTOX genes were generally lower in the high inflammation endotype at T0 and increased significantly by T1, when compared with low inflammation endotype within the PCV13 group (Fig 9H-9I).

Figure 9: Clinical and immunological variables associated with serotype-specific IgG responses

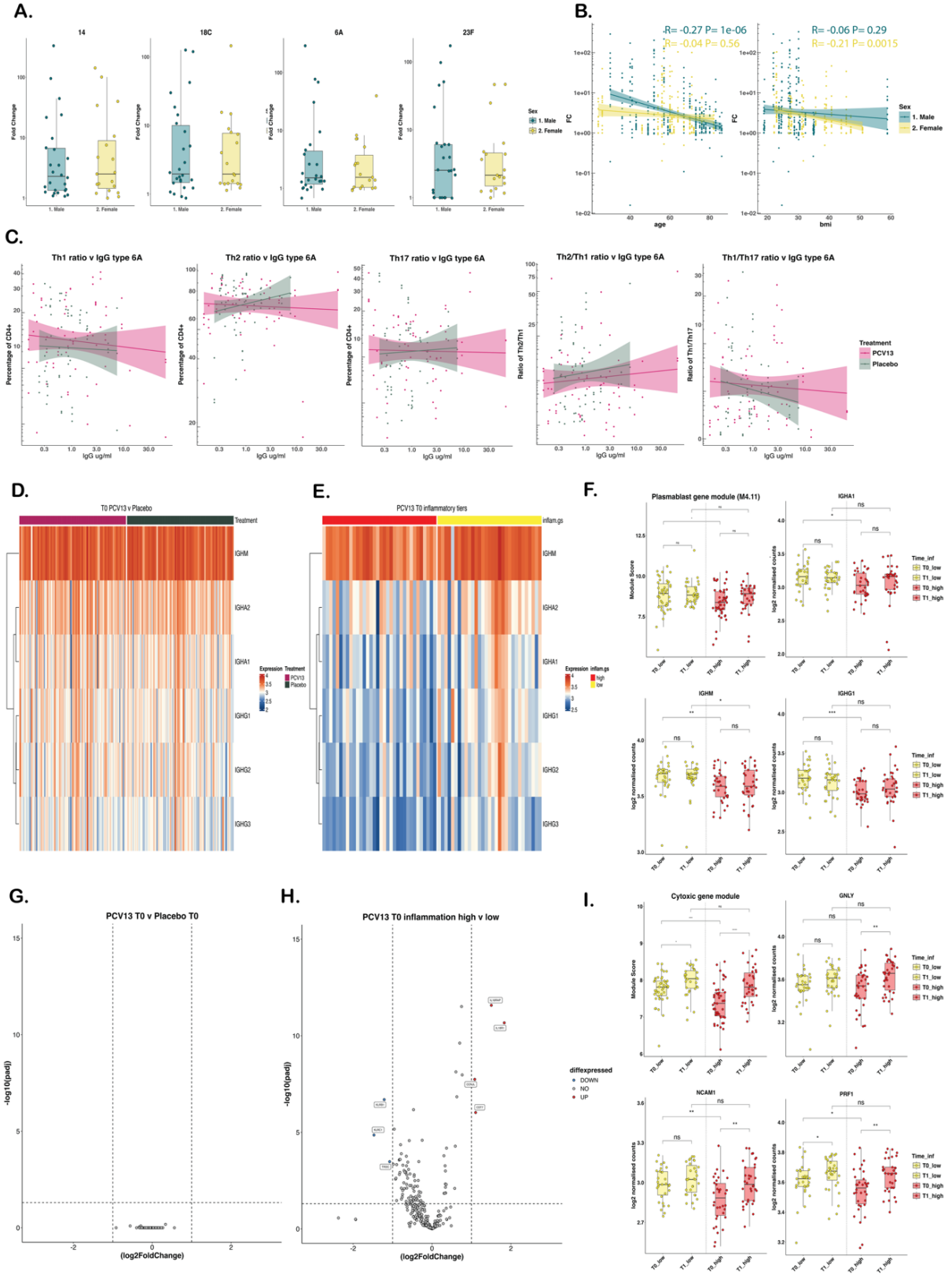


Fig.9A: Box and whisker plots of fold change for serotypes 14, 18C, 6A and 23F, split by male and female. **Fig.9B:** Fold change, split by sex plotted against age and BMI showing coefficient of

determination (R) and p value for males and females. **Fig.9C:** Correlation plot showing lack of statistically significant association between serotype-specific IgG (6A as example) with T helper subsets (T_H1, TH2, TH17) and their corresponding ratios (T_H2/ T_H1, T_H1/ T_H17). **Fig.9D:** No difference in immunoglobulin heavy chain genes (*IGHM, IGHG1, IGHG2, IGHG3, IGHA1, IGHA2*) between the PCV13 and placebo groups at baseline; **Fig.9E-9F:** Within the PCV13 group, inflammation high endotype had lower expression of immunoglobulin heavy chain genes and plasmablast module (4.11) scores at baseline, with no difference between T0 and T1 timepoints. **Fig.9G:** Volcano plot comparing the cytotoxic module genes between the PCV13 and placebo groups at baseline; **Fig.9H:** Within the PCV13 group, volcano plot comparing the cytotoxic module genes between inflammation high and low endotypes at baseline. **Fig.9I:** Within the PCV13 group, bar plots comparing the cytotoxic module genes between inflammation high and low endotypes at baseline, and between T0 and T1 timepoints. * = p<0.05; **=p<0.01; ***p<0.001 using non-parametric tests.

20. Conclusion

In this trial, involving adult sepsis survivors, PCV13 compared with placebo did not improve the clinical primary outcome of time to first infection-related rehospitalization or death at 365 days, with the 95% CI included both a potential reduction and an increase. The observed treatment effects were consistent across the secondary clinical outcomes. The significantly more reinfection events at 365 days observed in the PCV13 group (40 hospitalized, 30 community-treated) versus placebo (36 hospitalized, 26 community-treated), could either be a chance finding, or to the systemic effects of PCV13 vaccination treated as suspected infections as we used ICD-10 coding rather than microbiological confirmation of infection in our trial. Taken together, our clinical outcome results indicate the need to study how vaccines that are currently used as part of routine follow up care impact on long-term outcomes in sepsis survivor populations.

We also evaluated immunogenicity of an adjuvant vaccine (i.e., PCV13) in sepsis survivors. Our immunological analyses included blood samples post hospital discharge and shows that the sepsis survivors have evidence of inflammation, immunosuppression and ongoing innate and adaptive immune responses after hospital discharge. Despite the evidence of inflammation in sepsis survivors, PCV13 administration did not result in excessive cytokine responses. We observed heterogenous immunogenicity to PCV13 vaccination in sepsis survivors. This inference is supported by variations in serotype-specific IgG levels in the PCV13 group. PCV13 also increased B cell subsets like IgG memory and T cell subsets like T_H1 despite the pre-vaccination T_H2 skewing in the trial population, indicating T cell effects. We found differences in BTMs suggesting innate immune cell vaccine effects and T cell dependent responses. Fourati et al (29) reported high, mid and low pre-vaccination inflammatory endotypes, with the high inflammation endotype associated with greater IgG responses. In contrast, our pre-vaccination data suggested high and low inflammation endotypes that had heterogenous IgG responses as well other immune cell effects, without the distinct pattern reported by Fourati et al (29). We observed several differences from the previously described associations with PCV13 immunogenicity in a healthy adult cohort (30). Our immunological readouts highlight several

additional findings that inform future research. Sepsis survivors have ongoing immune impairments. Although T cell-dependent vaccine responses occur, vaccine immunogenicity is significantly heterogenous and altered in sepsis survivors. Currently, sepsis survivors receive the same routine vaccinations as non-sepsis patients. However, our results highlight the need for systematic research on vaccine immunogenicity in sepsis survivors to inform their ideal vaccination schedule.

Infection-related rehospitalizations in sepsis survivors could be either due to uncleared infection from the index sepsis admission or new infection (20). All vaccines have target pathogen-specific protective immunogenicity which, in our case, was PCV13 serotype-specific IgG, the magnitude of which was consistent with observations in healthy adults (54, 55). Given observations that T cell-dependent vaccine immunogenicity occurs in sepsis survivors, vaccines with heterologous immunogenicity (22, 23) such as Bacillus Calmette-Guérin (BCG) or live vaccines or a non-live recombinant glycoprotein E adjuvanted herpes zoster vaccines could be next steps in this research. The trained immunity effects of vaccines occur from the epigenetic modifications in immune cells that induce inflammatory responses to subsequent challenge with unrelated pathogens. However, such heterologous responses are often stochastic, vary by pathogen, BCG vaccine strains, and with previous exposure to BCG, among other factors. Thus, additional experimental work is required to identify patterns of response to BCG in sepsis survivors and the predictors of favourable responses, particularly as the heterologous BCG effects did not lower the risk of Covid-19 when compared with a placebo, in healthy population (56). Additionally, based on immune alterations described in our work, there is a need to explore prospective patient stratification, opportunities to improve either vaccine responsiveness, or to identify repurposed immunomodulators for future studies in sepsis survivors. By enriching the higher risk or higher response population of sepsis survivors with additional experimental research, such interventions could be made more precise.

Our trial has several strengths. The VACIRiSS trial was placebo-controlled with embedded biology and, to our knowledge, the first trial assessing efficacy and immunogenicity to any vaccine immunogenicity in sepsis survivors. Quadruple-blinding reduces bias, as the participants, all researchers including those completing follow-ups, data linkages, and biological measurements were blinded to treatment allocation, with independent WHO reference lab measuring PCV13 immunogenicity. Our immunological analyses provide a comprehensive picture of the altered immune state in sepsis survivors and indicate a need for future research to target the immune mechanisms with vaccine and immunomodulators to improve re-infection outcomes in sepsis survivors.

Our trial has limitations. As there were no previous estimates for expected treatment effects in sepsis survivors to inform our sample size calculations, the effect size used in our calculations and observed confidence intervals precludes excluding a relatively small benefit or harm. The primary site of infections could influence subsequent reinfections(31, 57), which we accounted for by adjusting with SSIP score. Balanced baseline characteristics are not a pre-requisite for valid inference in a well conducted randomized controlled trial as any random variability is captured in the uncertainty boundaries of the final estimate(58). The low proportion of randomized participants

relative to the number screened raises potential concerns around external validity. However, our exclusion criteria were specifically designed to exclude patients with established indications for pneumococcal vaccines (such as chemotherapy, splenectomy). These patients represent most of the patients excluded; therefore, this is unlikely to be an issue. This also highlights the prevalent comorbidity burden in sepsis survivors, consistent with the literature(3, 5, 7, 57). We did not evaluate the opsonophagocytic capacity(55) of serotype-specific IgG. We neither limited other vaccines participants could receive during the follow-up period nor study vaccine-vaccine interactions (31, 59). We did not evaluate the impact of PCV13 on the development of trained immunity, an innate immune memory mechanism with features such as monocyte reprogramming and epigenetic alterations (21). We did not evaluate the incidence of pneumococcal infection in our trial population, as our hypothesis was that PCV13 would reduce the entire burden of infections due to potential effects from the adjuvant in PCV13. Sepsis survivors have increased susceptibility to other non-infection outcomes than infections such as cardiovascular complications. Although we did not consider such long-term outcomes as secondary outcomes, we report the rehospitalization as MedDRA Code Body Systems adverse events that captures such outcomes.

In summary, while PCV13 demonstrated immunogenicity in adult sepsis survivors, it did not improve the primary clinical outcome of time to the first infection-related rehospitalization or death at 365 days. Given our clinical and immunological findings, future research is urgently needed to understand the translatability of vaccines given during follow up and the potential role of other immunomodulators in improving outcomes in sepsis survivors.

21. Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 15/Oct/2025.

22. References

1. M. Singer, C. S. Deutschman, C. W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G. R. Bernard, J. D. Chiche, C. M. Coopersmith, R. S. Hotchkiss, M. M. Levy, J. C. Marshall, G. S. Martin, S. M. Opal, G. D. Rubenfeld, T. van der Poll, J. L. Vincent, D. C. Angus, The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 315, 801–810 (2016).
2. K. E. Rudd, S. C. Johnson, K. M. Agesa, K. A. Shackelford, D. Tsoi, D. R. Kievlan, D. V. Colombara, K. S. Ikuta, N. Kissoon, S. Finfer, C. Fleischmann-Struzek, F. R. Machado, K. K. Reinhart, K. Rowan, C. W. Seymour, R. S. Watson, T. E. West, F. Marinho, S. I. Hay, R. Lozano, A. D. Lopez, D. C. Angus, C. J. L. Murray, M. Naghavi, Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 395, 200–211 (2020).
3. H. C. Prescott, D. C. Angus, Enhancing Recovery From Sepsis: A Review. JAMA 319, 62–75 (2018).
4. M. Shankar-Hari, G. D. Rubenfeld, Understanding Long-Term Outcomes Following Sepsis: Implications
and Challenges. Curr. Infect. Dis. Rep. 18, 37 (2016).
5. M. Shankar-Hari, M. Ambler, V. Mahalingasivam, A. Jones, K. Rowan, G. D. Rubenfeld, Evidence for a causal link between sepsis and long-term mortality: a systematic review of epidemiologic studies. Crit. Care 20, 101 (2016).
6. M. Shankar-Hari, R. Saha, J. Wilson, H. C. Prescott, D. Harrison, K. Rowan, G. D. Rubenfeld, N. K. J. Adhikari, Rate and risk factors for rehospitalisation in sepsis survivors: systematic review and meta-analysis. Intensive Care Med. 46, 619–636 (2020).
7. H. C. Prescott, T. J. Iwashyna, B. Blackwood, T. Calandra, L. L. Chlan, K. Choong, B. Connolly, P. Dark, L. Ferrucci, S. Finfer, T. D. Girard, C. Hodgson, R. O. Hopkins, C. L. Hough, J. C. Jackson, F. R. Machado, J. C. Marshall, C. Misak, D. M. Needham, P. Panigrahi, K. Reinhart, S. Yende, R. Zafonte, K. M. Rowan, D. C. Angus, Understanding and Enhancing Sepsis Survivorship. Priorities for Research and Practice. Am. J. Respir. Crit. Care Med. 200, 972–981 (2019).
8. A. Beane, M. Shankar-Hari, Long-term ill health in sepsis survivors: an ignored health-care challenge? Lancet 404, 1178–1180 (2024).
9. S. Yende, J. A. Kellum, V. B. Talisa, O. M. Peck Palmer, C. H. Chang, M. R. Filbin, N. I. Shapiro, P. C. Hou, A. Venkat, F. LoVecchio, K. Hawkins, E. D. Crouser, A. B. Newman, D. C. Angus, Long-term Host Immune Response Trajectories Among Hospitalized Patients With Sepsis. JAMA Netw Open 2, e198686 (2019).
10. C. Arens, S. A. Bajwa, C. Koch, B. H. Siegler, E. Schneck, A. Hecker, S. Weiterer, C. Lichtenstern, M. A. Weigand, F. Uhle, Sepsis-induced long-term immune paralysis--results of a descriptive, explorative study. Crit. Care 20, 93 (2016).
11. D. B. Darden, G. L. Ghita, Z. Wang, J. A. Stortz, M. C. Lopez, M. C. Cox, R. B. Hawkins, J. C. Rincon, L. S. Kelly, B. P. Fenner, T. Ozrazgat-Baslanti, C. Leeuwenburgh, A. Bihorac, T. J. Loftus, F. A. Moore, S. C. Brakenridge, H. V. Baker, R. Bacher, A. M. Mohr, L. L. Moldawer, P. A. Efron, Chronic Critical Illness Elicits a Unique Circulating Leukocyte Transcriptome in Sepsis Survivors. J Clin Med 10, (2021).
12. P. A. Assis, R. M. Allen, M. A. Schaller, S. L. Kunkel, J. R. Bermick, Metabolic reprogramming and dysregulated IL-17 production impairs CD4 T cell function post sepsis. iScience 27, 110114 (2024).

13. T. van der Poll, M. Shankar-Hari, W. J. Wiersinga, The immunology of sepsis. Immunity 54, 245047 –2464 (2021).
14. S. Cajander, M. Kox, B. P. Scicluna, M. A. Weigand, R. A. Mora, S. B. Flohe, I. Martin-Loeches, G. Lachmann, M. Girardis, A. Garcia-Salido, F. M. Brunkhorst, M. Bauer, A. Torres, A. Cossarizza, G. Monneret, J. M. Cavaillon, M. Shankar-Hari, E. J. Giamarellos-Bourboulis, M. S. Winkler, T. Skirecki, M. Osuchowski, I. Rubio, J. F. Bermejo-Martin, J. C. Schefold, F. Venet, Profiling the dysregulated immune response in sepsis: overcoming challenges to achieve the goal of precision medicine. The Lancet. Respiratory medicine 12, 305–322 (2024).
15. M. Shankar-Hari, T. Calandra, M. P. Soares, M. Bauer, W. J. Wiersinga, H. C. Prescott, J. C. Knight, K. J. Baillie, L. D. J. Bos, L. P. G. Derde, S. Finfer, R. S. Hotchkiss, J. Marshall, P. J. M. Openshaw, C. W. Seymour, F. Venet, J. L. Vincent, C. Le Tourneau, A. H. Maitland-van der Zee, I. B. McInnes, T. van der Poll, Reframing sepsis immunobiology for translation: towards informative subtyping and targeted immunomodulatory therapies. The Lancet. Respiratory medicine 12, 323–336 (2024).
16. R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson, R. E. Schmieg, Jr., J. J. Hui, K. C. Chang, D. F. Osborne, B. D. Freeman, J. P. Cobb, T. G. Buchman, I. E. Karl, Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans. J. Immunol. 166, 6952–6963 (2001).
17. M. Shankar-Hari, D. Fear, P. Lavender, T. Mare, R. Beale, C. Swanson, M. Singer, J. Spencer, Activation-Associated Accelerated Apoptosis of Memory B Cells in Critically Ill Patients With Sepsis. Crit. Care Med. 45, 875–882 (2017).
18. J. Choutka, V. Jansari, M. Hornig, A. Iwasaki, Unexplained post-acute infection syndromes. Nat. Med. 28, 911–923 (2022).
19. E. C. van der Slikke, A. Y. An, R. E. W. Hancock, H. R. Bouma, Exploring the pathophysiology of post-sepsis syndrome to identify therapeutic opportunities. EBioMedicine 61, 103044 (2020).
20. A. J. Pollard, E. M. Bijker, A guide to vaccinology: from basic principles to new developments. Nat. Rev. Immunol. 21, 83–100 (2021).
21. M. G. Netea, J. Dominguez-Andres, L. B. Barreiro, T. Chavakis, M. Divangahi, E. Fuchs, L. A. B. Joosten, J. W. M. van der Meer, M. M. Mhlanga, W. J. M. Mulder, N. P. Riksen, A. Schlitzer, J. L. Schultze, C. Stabell Benn, J. C. Sun, R. J. Xavier, E. Latz, Defining trained immunity and its role in health and disease. Nat. Rev. Immunol. 20, 375–388 (2020).
22. M. Saadatian-Elahi, P. Aaby, F. Shann, M. G. Netea, O. Levy, J. Louis, V. Picot, M. Greenberg, W. Warren, Heterologous vaccine effects. Vaccine 34, 3923–3930 (2016).
23. H. S. Goodridge, S. S. Ahmed, N. Curtis, T. R. Kollmann, O. Levy, M. G. Netea, A. J. Pollard, R. van Crevel, C. B. Wilson, Harnessing the beneficial heterologous effects of vaccination. Nat. Rev. Immunol. 16, 392–400 (2016).
24. E. Chilson, D. A. Scott, B. Schmoele-Thoma, W. Watson, M. M. Moran, R. Isturiz, Immunogenicity and safety of the 13-valent pneumococcal conjugate vaccine in patients with immunocompromising conditions: a review of available evidence. Hum. Vaccin. Immunother. 16, 2758–2772 (2020).
25. A. Lopez, X. Mariette, H. Bachelez, A. Belot, B. Bonnotte, E. Hachulla, M. Lahfa, O. Lortholary, P. Loulergue, S. Paul, X. Roblin, J. Sibilia, M. Blum, S. Danese, S. Bonovas, L. Peyrin-Biroulet, Vaccination recommendations for the adult immunosuppressed patient: A systematic review and comprehensive field synopsis. J. Autoimmun. 80, 10–27 (2017).

26. L. G. Rubin, M. J. Levin, P. Ljungman, E. G. Davies, R. Avery, M. Tomblyn, A. Bousvaros, S. Dhanireddy, L. Sung, H. Keyserling, I. Kang, A. Infectious Diseases Society of, 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin. Infect. Dis. 58, e44–100 (2014).
27. E. A. Clutterbuck, R. Lazarus, L. M. Yu, J. Bowman, E. A. Bateman, L. Diggle, B. Angus, T. E. Peto, P. C. Beverley, D. Mant, A. J. Pollard, Pneumococcal conjugate and plain polysaccharide vaccines have divergent effects on antigen-specific B cells. J. Infect. Dis. 205, 1408–1416 (2012).
28. S. Sterrett, B. J. Peng, R. L. Burton, D. C. LaFon, A. O. Westfall, S. Singh, M. Pride, A. S. Anderson, G. C. Ippolito, H. W. Schroeder, Jr., M. H. Nahm, A. Krishna Prasad, P. Goepfert, A. Bansal, Peripheral CD4 T follicular cells induced by a conjugated pneumococcal vaccine correlate with enhanced opsonophagocytic antibody responses in younger individuals. Vaccine 38, 1778–1786 (2020).
29. S. Fourati, L. E. Tomalin, M. P. Mule, D. G. Chawla, B. Gerritsen, D. Rychkov, E. Henrich, H. E. R. Miller, T. Hagan, J. Diray-Arce, P. Dunn, C. Human Immunology Project, O. Levy, R. Gottardo, M. M. Sarwal, J. S. Tsang, M. Suarez-Farinas, B. Pulendran, S. H. Kleinstein, R. P. Sekaly, Pan-vaccine analysis reveals innate immune endotypes predictive of antibody responses to vaccination. Nat. Immunol. 23, 1777 –1787 (2022).
30. S. Ravichandran, F. Erra-Diaz, O. E. Karakaslar, R. Marches, L. Kenyon-Pesce, R. Rossi, D. Chaussabel, D. Nehar-Belaid, D. C. LaFon, V. Pascual, K. Palucka, S. Paust, M. H. Nahm, G. A. Kuchel, J. Banchereau, D. Ucar, Distinct baseline immune characteristics associated with responses to conjugated and unconjugated pneumococcal polysaccharide vaccines in older adults. Nat. Immunol. 25, 316–329 (2024).
31. M. Shankar-Hari, G. D. Rubenfeld, P. Ferrando-Vivas, D. A. Harrison, K. Rowan, Development, Validation, and Clinical Utility Assessment of a Prognostic Score for 1-Year Unplanned Rehospitalization or Death of Adult Sepsis Survivors. JAMA Netw Open 3, e2013580 (2020).
32. T. Hagan, B. Gerritsen, L. E. Tomalin, S. Fourati, M. P. Mule, D. G. Chawla, D. Rychkov, E. Henrich, H. E. R. Miller, J. Diray-Arce, P. Dunn, A. Lee, C. Human Immunology Project, O. Levy, R. Gottardo, M. M. Sarwal, J. S. Tsang, M. Suarez-Farinas, R. P. Sekaly, S. H. Kleinstein, B. Pulendran, Transcriptional atlas of the human immune response to 13 vaccines reveals a common predictor of vaccine-induced antibody responses. Nat. Immunol. 23, 1788–1798 (2022).
33. S. Li, N. Rouphael, S. Duraisingham, S. Romero-Steiner, S. Presnell, C. Davis, D. S. Schmidt, S. E. Johnson, A. Milton, G. Rajam, S. Kasturi, G. M. Carlone, C. Quinn, D. Chaussabel, A. K. Palucka, M. J. Mulligan, R. Ahmed, D. S. Stephens, H. I. Nakaya, B. Pulendran, Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat. Immunol. 15, 195 –204 (2014).
34. A. M. M. van Deursen, M. A. van Houten, C. Webber, M. Patton, D. A. Scott, S. Patterson, M. Sidhu, W. Drews, W. C. Gruber, E. A. Emini, D. E. Grobbee, M. J. M. Bonten, E. A. M. Sanders, Immunogenicity of the 13-Valent Pneumococcal Conjugate Vaccine in Older Adults With and Without Comorbidities in the Community-Acquired Pneumonia Immunization Trial in Adults (CAPiTA). Clin. Infect. Dis. 65, 787 –795 (2017).
35. https://www.ucl.ac.uk/impact/case-studies/2014/dec/evaluating-and-introducing-pneumococcal-conjugate - vaccines-uk-infant

36. S. N. Crooke, I. G. Ovsyannikova, G. A. Poland, R. B. Kennedy, Immunosenescence and human vaccine immune responses. Immun. Ageing 16, 25 (2019).
37. S. T. Chang, Y. F. Chuang, A. H. Li, Y. T. Fan, M. R. Liao, I. Y. Chen, R. W. Hung, T. O. Yang, Y. L. Chiu, Age-dependent immune profile in healthy individuals: an original study, systematic review and meta - analysis. Immun. Ageing 21, 75 (2024).
38. D. L. Gupta, S. Bhoi, T. Mohan, S. Galwnkar, D. N. Rao, Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with post traumatic sepsis. Cytokine 88, 214–221 (2016).
39. M. Xue, J. Xie, L. Liu, Y. Huang, F. Guo, J. Xu, Y. Yang, H. Qiu, Early and dynamic alterations of Th2/Th1 in previously immunocompetent patients with community-acquired severe sepsis: a prospective observational study. J. Transl. Med. 17, 57 (2019).
40. F. Venet, A. Pachot, A. L. Debard, J. Bohe, J. Bienvenu, A. Lepape, G. Monneret, Increased percentage of CD4+CD25+ regulatory T cells during septic shock is due to the decrease of CD4+CD25- lymphocytes. Crit. Care Med. 32, 2329–2331 (2004).
41. G. Monneret, A. L. Debard, F. Venet, J. Bohe, O. Hequet, J. Bienvenu, A. Lepape, Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. Crit. Care Med. 31, 2068–2071 (2003).
42. H. T. Maecker, J. P. McCoy, R. Nussenblatt, Standardizing immunophenotyping for the Human Immunology Project. Nat. Rev. Immunol. 12, 191–200 (2012).
43. M. A. Berkowska, G. J. Driessen, V. Bikos, C. Grosserichter-Wagener, K. Stamatopoulos, A. Cerutti, B. He, K. Biermann, J. F. Lange, M. van der Burg, J. J. van Dongen, M. C. van Zelm, Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. Blood 118, 2150–2158 (2011).
44. M. Fish, K. Arkless, A. Jennings, J. Wilson, M. J. Carter, G. Arbane, S. Campos, N. Novellas, R. Wester, N. Petrov, U. Niazi, B. Sanderson, R. Ellis, M. Saqi, J. Spencer, M. Singer, R. T. Martinez-Nunez, S. Pitchford, C. M. Swanson, M. Shankar-Hari, Cellular and molecular mechanisms of IMMunE dysfunction and Recovery from SEpsis-related critical illness in adults: An observational cohort study (IMMERSE) protocol paper. J Intensive Care Soc 23, 318–324 (2022).
45. I. Pedroza-Pacheco, A. J. McMichael, Immune signature atlas of vaccines: learning from the good responders. Nat. Immunol. 23, 1654–1656 (2022).
46. F. B. Vincent, D. Saulep-Easton, W. A. Figgett, K. A. Fairfax, F. Mackay, The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine Growth Factor Rev. 24, 203 – 215 (2013).
47. L. Lorente, M. M. Martín, N. Varo, J. M. Borreguero-León, J. Solé-Violán, J. Blanquer, L. Labarta, C. Díaz, A. Jiménez, E. Pastor, F. Belmonte, J. Orbe, J. A. Rodríguez, E. Gómez-Melini, J. M. Ferrer-Agüero, J. Ferreres, M. C. Llimiñana, J. A. Páramo, Association between serum soluble CD40 ligand levels and mortality in patients with severe sepsis. Crit. Care 15, R97 (2011).
48. A. Sinistro, C. Almerighi, C. Ciaprini, S. Natoli, E. Sussarello, S. D. Fino, F. Calò-Carducci, G. Rocchi, A. Bergamini, Downregulation of CD40 Ligand Response in Monocytes from Sepsis Patients. Clin. Vaccine Immunol. 15, 1851–1858 (2008).
49. W. Xu, H. Joo, S. Clayton, M. Dullaers, M. C. Herve, D. Blankenship, M. T. De La Morena, R. Balderas, C. Picard, J. L. Casanova, V. Pascual, S. Oh, J. Banchereau, Macrophages induce

differentiation of plasma cells through CXCL10/IP-10. J. Exp. Med. 209, 1813–1823, s1811–1812 (2012).
50. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014).
51. W. N. Haining, Strength in numbers: comparing vaccine signatures the modular way. Nat. Immunol. 15, 139–141 (2014).
52. L. H. Hartwell, J. J. Hopfield, S. Leibler, A. W. Murray, From molecular to modular cell biology. Nature 402, C47–52 (1999).
53. G. Yaari, C. R. Bolen, J. Thakar, S. H. Kleinstein, Quantitative set analysis for gene expression: a method to quantify gene set differential expression including gene-gene correlations. Nucleic Acids Res. 41, e170 (2013).
54. D. Goldblatt, B. D. Plikaytis, M. Akkoyunlu, J. Antonello, L. Ashton, M. Blake, R. Burton, R. Care, N. Durant, I. Feavers, P. Fernsten, F. Fievet, P. Giardina, K. Jansen, L. Katz, L. Kierstead, L. Lee, J. Lin, J. Maisonneuve, M. H. Nahm, J. Raab, S. Romero-Steiner, C. Rose, D. Schmidt, J. Stapleton, G. M. Carlone, Establishment of a new human pneumococcal standard reference serum, 007sp. Clin. Vaccine Immunol. 18, 1728–1736 (2011).
55. E. Mitsi, D. McLenaghan, A. S. Wolf, S. Jones, A. M. Collins, A. D. Hyder-Wright, D. Goldblatt, R. S. Heyderman, S. B. Gordon, D. M. Ferreira, Thirteen-Valent Pneumococcal Conjugate Vaccine-Induced Immunoglobulin G (IgG) Responses in Serum Associated With Serotype-Specific IgG in the Lung. J. Infect. Dis. 225, 1626–1631 (2022).
56. L. F. Pittet, N. L. Messina, F. Orsini, C. L. Moore, V. Abruzzo, S. Barry, R. Bonnici, M. Bonten, J. Campbell, J. Croda, M. Dalcolmo, K. Gardiner, G. Gell, S. Germano, A. Gomes-Silva, C. Goodall, A. Gwee, T. Jamieson, B. Jardim, T. R. Kollmann, M. V. G. Lacerda, K. J. Lee, M. Lucas, D. J. Lynn, L. Manning, H. S. Marshall, E. McDonald, C. F. Munns, S. Nicholson, A. O'Connell, R. D. d. Oliveira, S. Perlen, K. P. Perrett, C. Prat-Aymerich, P. C. Richmond, J. Rodriguez-Baño, G. d. Santos, P. V. d. Silva, J. W. Teo, P. Villanueva, A. Warris, N. J. Wood, A. Davidson, N. Curtis, Randomized Trial of BCG Vaccine to Protect against Covid-19 in Health Care Workers. N. Engl. J. Med. 388, 1582–1596 (2023).
57. K. M. DeMerle, S. C. Royer, M. E. Mikkelsen, H. C. Prescott, Readmissions for Recurrent Sepsis: New or Relapsed Infection? Crit. Care Med. 45, 1702–1708 (2017).
58. S. Senn, Seven myths of randomisation in clinical trials. Stat. Med. 32, 1439–1450 (2013).
59. E. Rezel-Potts, M. C. Gulliford, A. B. S. G. Safe, Sepsis recording in primary care electronic health records, linked hospital episodes and mortality records: Population-based cohort study in England. PLoS One 15, e0244764 (2020).
60. https://www.nhs.uk/vaccinations/pneumococcal-vaccine/; Accessed on 22-July-2024
61. R. J. Little, R. J. Lewis, Estimands, Estimators, and Estimates. JAMA 326, 967–968 (2021).
62. https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics
63. K. Rufibach, Treatment effect quantification for time-to-event endpoints-Estimands, analysis strategies, and beyond. Pharm. Stat. 18, 145–165 (2019).
64. https://www.biolegend.com/Files/Images/BioLegend/legendplex/manuals/LEGENDplex_Data_Analysis_Software_v8.0_User_Guide.pdf ; Date Accessed July 29 2024

65. https://www.qiagen.com/us/resources/resourcedetail?id=9a4cdd1346 -8b72-4b33-877cba60a83b87be&
lang=en
66. https://www.cancergenomicscloud.org/tools-and-resources
67. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120 (2014).
68. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford, Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 14, 417–419 (2017).
69. P. Ewels, M. Magnusson, S. Lundin, M. Kaller, MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32, 3047–3048 (2016).
70. C. Soneson, M. I. Love, M. D. Robinson, Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res 4, 1521 (2015).
71. https://github.com/shuzhao-li/BTM
72. A. Liberzon, C. Birger, H. Thorvaldsdottir, M. Ghandi, J. P. Mesirov, P. Tamayo, The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 1, 417–425 (2015).
73. Shankar-Hari M, Rubenfeld GD, Ferrando-Vivas P, Harrison DA, Rowan K. Development, Validation, and Clinical Utility Assessment of a Prognostic Score for 1-Year Unplanned Rehospitalization or Death of Adult Sepsis Survivors. JAMA Netw Open. Sep 1 2020;3(9):e2013580. doi:10.1001/jamanetworkopen.2020.13580
74. Rufibach K. Treatment effect quantification for time-to-event endpoints-Estimands, analysis strategies, and beyond. Pharm Stat. Mar 2019;18(2):145-165. doi:10.1002/pst.1917

APPENDICES

i) Summary of treatment-emergent AEs in the per protocol population

System Organ Class	Preferred Term	Number of Subjects Experiencing the AE in Active Arm	Total Number of Occurrences of the AE	Number of Subjects Experiencing the AE in Placebo Arm	Total Number of Occurrences of the AE
Blood and lymphatic system disorders	-				
Cardiac disorders	-				
Congenital, familial and genetic disorders	-				
Ear and labyrinth disorders	-				
Eye Disorders	-				
Gastrointestinal disorders	Abdominal pain	0 (0.00)	0	1 (0.95)	1
	Diarrhoea	0 (0.00)	0	1 (0.95)	1
	Taste disorder	0 (0.00)	0	1 (0.95)	1
	Vomiting	0 (0.00)	0	1 (0.95)	1
General disorders and administration site conditions	Impaired healing	1 (0.99)	1	0 (0.00)	0
	Injection site pain	1 (0.99)	1	0 (0.00)	0
	Joint range of motion decreased	0 (0.00)	0	2 (1.90)	2
	Pyrexia	1 (0.99)	1	2 (1.90)	2

Hepatobiliary disorders	-				
Immune system disorders	-				
Infections and infestations	Biliary sepsis	0 (0.00)	0	1 (0.95)	1
	Cellulitis	1 (0.99)	1	2 (1.90)	2
	Ear infection	1 (0.99)	1	1 (0.95)	1
	Epididymitis	1 (0.99)	1	0 (0.00)	0
	Gastrointestinal infection	1 (0.99)	1	0 (0.00)	0
	Genitourinary tract infection	0 (0.00)	0	1 (0.95)	1
	Herpes zoster	0 (0.00)	0	1 (0.95)	1
	Infected bite	0 (0.00)	0	2 (1.90)	2
	Infection	3 (2.97)	6	2 (1.90)	3
	Infective exacerbation of chronic obstructive airways disease	0 (0.00)	0	2 (1.90)	6
	Localised infection	5 (4.95)	5	2 (1.90)	2
	Lower respiratory tract infection	4 (3.96)	7	6 (5.71)	7
	Mycoplasma infection	0 (0.00)	0	1 (0.95)	1
	Oral infection	1 (0.99)	1	0 (0.00)	0
	Pharyngitis	0 (0.00)	0	1 (0.95)	1
	Pneumonia	3 (2.97)	3	0 (0.00)	0
	Respiratory tract infection	1 (0.99)	1	2 (1.90)	3
	Tonsillitis	1 (0.99)	1	1 (0.95)	1
	Tracheostomy infection	0 (0.00)	0	1 (0.95)	1
	Upper respiratiry tract infection	1 (0.99)	1	0 (0.00)	0
	Urinary tract infection	7 (6.93)	7	4 (3.81)	10
	Vascular device infection	1 (0.99)	1	0 (0.00)	0

	Wound infection	0 (0.00)	0	1 (0.95)	1
Injury, poisoning and procedural complications	Fall	1 (0.99)	1	1 (0.95)	1
Investigations	Ureteroscopy	0 (0.00)	0	1 (0.95)	1
Metabolism and nutritional disorders	Hyponatraemia	0 (0.00)	0	1 (0.95)	1
	Malnutrition	0 (0.00)	0	1 (0.95)	1
Musculoskeletal and connective tissue disorders	Myalgia	0 (0.00)	0	1 (0.95)	1
	Pain in extremity	1 (0.99)	1	1 (0.95)	1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Bartholin's cyst	1 (0.99)	1	0 (0.00)	0
Nervous system disorders	Bell's palsy	1 (0.99)	1	0 (0.00)	0
	Headache	0 (0.00)	0	2 (1.90)	2
Pregnancy, puerperium and perinatal conditions	-				
Product issues	-				
Psychiatric disorders	Mood altered	0 (0.00)	0	1 (0.95)	1
Renal and urinary disorders	-				
Reproductive system and breast disorders	-				
Respiratory, thoracic and mediastinal disorders	Cough	0 (0.00)	0	1 (0.95)	1
	Dyspnoea	1 (0.99)	1	0 (0.00)	0
Skin and subcutaneous tissue disorders	Acne	0 (0.00)	0	1 (0.95)	1

	Actinic keratosis	0 (0.00)	0	1 (0.95)	1
	Rash	1 (0.99)	1	0 (0.00)	0
	Skin ulcer	0 (0.00)	0	2 (1.90)	2
Social circumstances	-				
Surgical and medical procedures	Enterostomy	0 (0.00)	0	1 (0.95)	1
	Hospitalisation	1 (0.99)	1	0 (0.00)	0
	Immunisation	0 (0.00)	0	1 (0.95)	1
Vascular disorders	-				

ii) Summary of treatment-emergent ARs in the per protocol population

System Organ Class	Preferred Term	Number of Subjects Experiencing the AR in Active Arm	Total Number of Occurrences of the AR	Number of Subjects Experiencing the AR in Placebo Arm	Total Number of Occurrences of the AR
Blood and lymphatic system disorders	-				
Cardiac disorders	-				
Congenital, familial and genetic disorders	-				
Ear and labyrinth disorders	-				
Eye Disorders	-				
Gastrointestinal disorders	Nausea	0 (0.00)	0	1 (0.95)	1
	Vomiting	0 (0.00)	0	1 (0.95)	1
General disorders and	Injection site bruising	1 (0.99)	1	1 (0.95)	1

administration site conditions					
	Injection site pain	7 (6.93)	7	5 (4.76)	6
	Injection site swelling	2 (1.98)	2	2 (1.90)	2
	Joint range of motion decreased	2 (1.98)	2	1 (0.95)	1
	Pyrexia	4 (3.96)	4	3 (2.86)	3
Hepatobiliary disorders	-				
Immune system disorders	-				
Infections and infestations	Lower respiratory tract infection	1 (0.99)	1	0 (0.00)	0
Injury, poisoning and procedural complications	-				
Investigations	-				
Metabolism and nutritional disorders	-				
Musculoskeletal and connective tissue disorders	Myalgia	5 (4.95)	6	0 (0.00)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	-				
Nervous system disorders	Headache	3 (2.97)	3	4 (3.81)	4
Pregnancy, puerperium and perinatal conditions	-				
Product issues	-				
Psychiatric disorders	-				
Renal and urinary disorders	-				

Reproductive system and breast disorders	-				
Respiratory, thoracic and mediastinal disorders	-				
Skin and subcutaneous tissue disorders	-				
Social circumstances	-				
Surgical and medical procedures	-				
Vascular disorders	-				

iii) Summary of treatment-emergent SAEs in the study population

System Organ Class	Preferred Term	Number of Participants Experiencing the SAE in Active Arm	Total Number of Occurrences of the SAE	Number of Participants Experiencing the SAE in Placebo Arm	Total Number of Occurrences of the SAE
Blood and lymphatic system disorders	-				
Cardiac disorders	Acute myocardial infarction	0 (0.00)	0	1 (0.91)	2
	Acute pulmonary oedema	1 (0.96)	1	2 (1.82)	2
	Cardiac failure	0 (0.00)	0	1 (0.91)	1
	Cardiac failure acute	0 (0.00)	0	1 (0.91)	1
	Chest pain	1 (0.96)	1	1 (0.91)	1
	Coronary artery disease	1 (0.96)	1	0 (0.00)	0

	Hypervolaemia	1 (0.96)	1	0 (0.00)	0
	Pericardial effusion	1 (0.96)	1	0 (0.00)	0
	Tachyarrhythmia	1 (0.96)	1	0 (0.00)	0
Congenital, familial and genetic disorders	-				
Ear and labyrinth disorders	-				
Eye Disorders	-				
Gastrointestinal disorders	Abdominal pain	2 (1.92)	2	0 (0.00)	0
	Abdominal wound dehiscence	1 (0.96)	1	0 (0.00)	0
	Gastrointestinal haemorrhage	2 (1.92)	2	0 (0.00)	0
	Haematemesis	0 (0.00)	0	1 (0.91)	1
	Hiatus hernia	1 (0.96)	1	0 (0.00)	0
	Intestinal obstruction	0 (0.00)	0	1 (0.91)	1
	Large intestine perforation	0 (0.00)	0	1 (0.91)	1
	Oesophageal ulcer	1 (0.96)	1	0 (0.00)	0
	Oesophagitis	0 (0.00)	0	1 (0.91)	1
	Pancreatitis	1 (0.96)	1	0 (0.00)	0
	Pancreatitis chronic	1 (0.96)	3	0 (0.00)	0
	Pancreatitis necrotising	1 (0.96)	2	0 (0.00)	0
	Small intestinal obstruction	4 (3.85)	4	0 (0.00)	0
	Upper gastrointestinal haemorrhage	1 (0.96)	1	0 (0.00)	0
	Varices oesophageal	0 (0.00)	0	1 (0.91)	1
	Vomiting	1 (0.96)	1	0 (0.00)	0

General disorders and administration site conditions	Death	3 (2.88)	3	1 (0.91)	1
	Feeding tube complication	1 (0.96)	2	0 (0.00)	0
	Hypothermia	0 (0.00)	0	1 (0.91)	1
	Pyrexia	3 (2.88)	3	0 (0.00)	0
Hepatobiliary disorders	Cholecystitis	1 (0.96)	2	1 (0.91)	1
	Hepatic cirrhosis	0 (0.00)	0	1 (0.91)	1
Immune system disorders	-				
Infections and infestations	Abdominal infection	1 (0.96)	1	1 (0.91)	1
	Arthritis bacterial	0 (0.00)	0	1 (0.91)	1
	Atypical pneumonia	0 (0.00)	0	1 (0.91)	1
	COVID-19	4 (3.85)	4	2 (1.82)	2
	Cellulitis	1 (0.96)	1	0 (0.00)	0
	Clostridium difficile infection	0 (0.00)	0	1 (0.91)	1
	Emphysematous cystitis	0 (0.00)	0	1 (0.91)	1
	Endocarditis	0 (0.00)	0	1 (0.91)	2
	Groin infection	0 (0.00)	0	1 (0.91)	1
	Infection	0 (0.00)	0	1 (0.91)	1
	Infective exacerbation of chronic obstructive airways disease	2 (1.92)	3	2 (1.82)	4
	Localised infection	1 (0.96)	1	0 (0.00)	0
	Lower respiratory tract infection	5 (4.81)	5	3 (2.73)	3
	Pelvic infection	1 (0.96)	1	0 (0.00)	0
	Pneumonia	6 (5.77)	9	8 (7.27)	8
	Pneumonia aspiration	0 (0.00)	0	2 (1.82)	2

	Postoperative wound infection	1 (0.96)	1	0 (0.00)	0
	Pseudoaneurysm infection	0 (0.00)	0	1 (0.91)	1
	Pyelonephritis	1 (0.96)	2	0 (0.00)	0
	Sepsis	3 (2.88)	3	2 (1.82)	2
	Skin infection	0 (0.00)	0	1 (0.91)	1
	Staphylococcal infection	0 (0.00)	0	1 (0.91)	1
	Urinary tract infection	2 (1.92)	2	2 (1.82)	2
	Urosepsis	0 (0.00)	0	1 (0.91)	1
	Vascular device infection	1 (0.96)	1	0 (0.00)	0
	Wound infection	1 (0.96)	1	1 (0.91)	1
Injury, poisoning and procedural complications	Animal bite	1 (0.96)	1	0 (0.00)	0
	Catheter site pain	1 (0.96)	1	0 (0.00)	0
	Fall	3 (2.88)	3	2 (1.82)	2
	Femur fracture	0 (0.00)	0	1 (0.91)	1
	Hip Fracture	1 (0.96)	1	0 (0.00)	0
	Overdose	1 (0.96)	1	0 (0.00)	0
	Post procedural complication	1 (0.96)	1	0 (0.00)	0
Investigations	Prostate examination	1 (0.96)	1	0 (0.00)	0
Metabolism and nutritional disorders	Dehydration	1 (0.96)	1	0 (0.00)	0
	Diabetic ketoacidosis	1 (0.96)	11	1 (0.91)	1
	Electrolyte imbalance	2 (1.92)	2	0 (0.00)	0
	Feeding intolerance	1 (0.96)	1	0 (0.00)	0
	Hyperglycaemia	1 (0.96)	1	0 (0.00)	0
Musculoskeletal and connective tissue	Osteoarthritis	0 (0.00)	0	1 (0.91)	1

disorders					
	Pain in extremity	1 (0.96)	1	0 (0.00)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Adenocarcinoma	0 (0.00)	0	1 (0.91)	1
Nervous system disorders	Cerebrovascular accident	2 (1.92)	2	1 (0.91)	1
	Confusional state	0 (0.00)	0	1 (0.91)	2
	Orthostatic hypotension	1 (0.96)	1	0 (0.00)	0
Pregnancy, puerperium and perinatal conditions	-				
Product issues	-				
Psychiatric disorders	-	1	1	0	0
Renal and urinary disorders	Chronic kidney disease	0 (0.00)	0	1 (0.91)	1
	Renal failure	1 (0.96)	1	0 (0.00)	0
	Urinary retention	1 (0.96)	2	0 (0.00)	0
Reproductive system and breast disorders	-				
Respiratory, thoracic and mediastinal disorders	Chest pain	1 (0.96)	1	0 (0.00)	0
	Chronic obstructive pulmonary disease	1 (0.96)	1	0 (0.00)	0
	Dyspnoea	1 (0.96)	1	1 (0.91)	1
	Hypoxia	1 (0.96)	1	0 (0.00)	0
	Pleural effusion	1 (0.96)	1	0 (0.00)	0
	Pleurisy	0 (0.00)	0	1 (0.91)	1
	Pneumothorax	1 (0.96)	1	1 (0.91)	3
	Pulmonary oedema	2 (1.92)	2	0 (0.00)	0
	Respiratory failure	1 (0.96)	1	1 (0.91)	1

Skin and subcutaneous tissue disorders	Decubitis ulcer	1 (0.96)	1	0 (0.00)	0
	Diabetic foot infection	1 (0.96)	1	1 (0.91)	1
	Hidradenitis	1 (0.96)	2	0 (0.00)	0
Social circumstances	-				
Surgical and medical procedures	Abscess drainage	0 (0.00)	0	1 (0.91)	1
	Aneurysm repair	0 (0.00)	0	1 (0.91)	1
	Bladder catheter removal	1 (0.96)	1	0 (0.00)	0
	Coronary artery surgery	0 (0.00)	0	1 (0.91)	1
	Debridement	1 (0.96)	2	0 (0.00)	0
	Hospitalisation	2 (1.92)	2	0 (0.00)	0
	Nephrectomy	0 (0.00)	0	1 (0.91)	1
	Pancreatic pseudocyst drainage	1 (0.96)	1	0 (0.00)	0
	Tracheal lesion excision	1 (0.96)	1	0 (0.00)	0
Vascular disorders	Haematoma	0 (0.00)	0	1 (0.91)	1
	Peripheral arterial occlusive disease	0 (0.00)	0	1 (0.91)	2
	Peripheral ischaemia	3 (2.88)	3	0 (0.00)	0

iv) Summary of treatment-emergent Serious Adverse Reactions (SARs) in the study population

No SARs were reported in the study population.

Fig.A1: PCV13 serotype specific antibodies and fold change

Fig.A1A-A1I: Box and Whisker plots showing median and inter quartile range in serotype-specific immunoglobulin G between T0 and T2 by allocation to PCV13 and placebo. * = p<0.05; **=p<0.01; ***p<0.001, ****p<0.0001 using non-parametric tests.

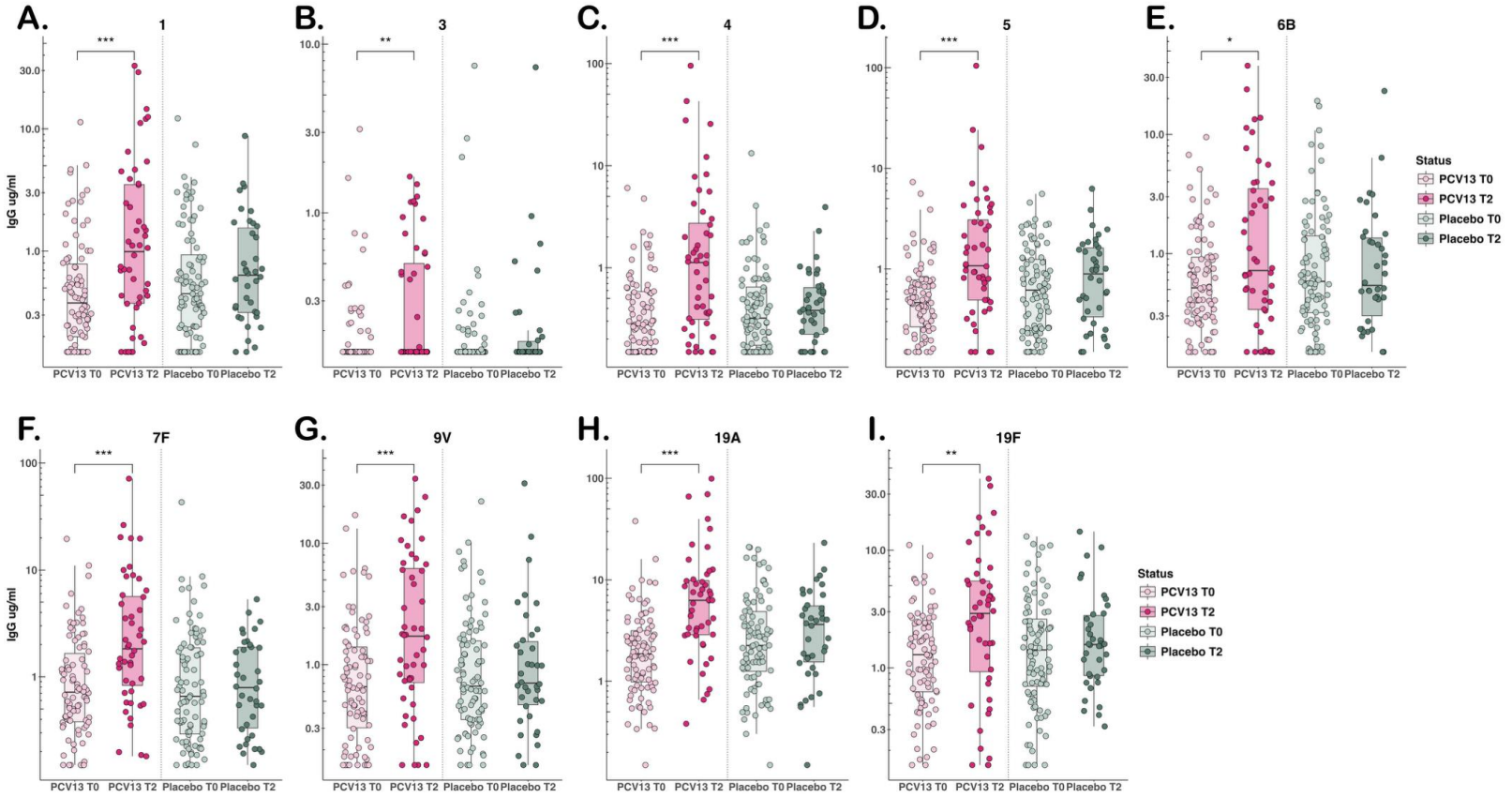
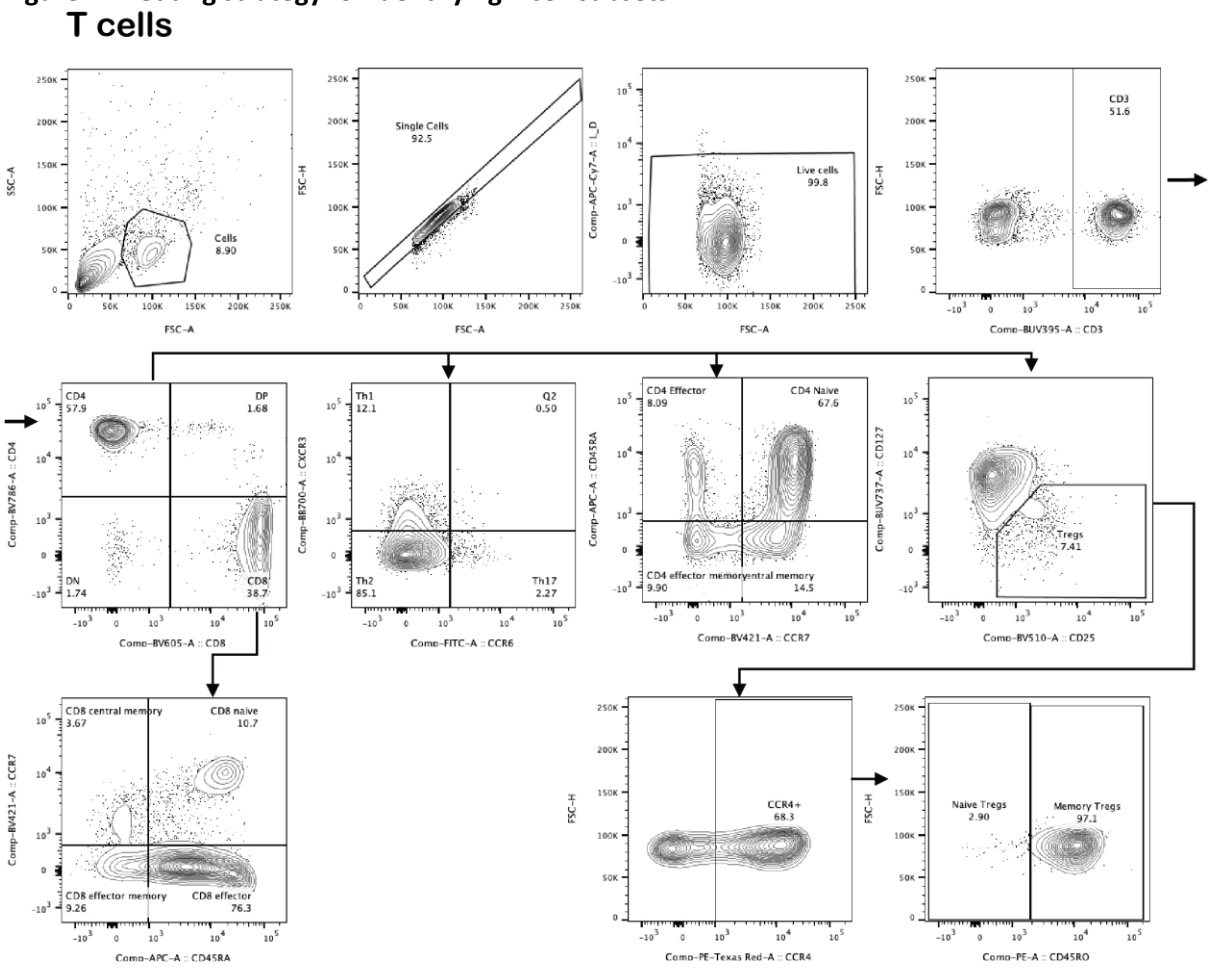
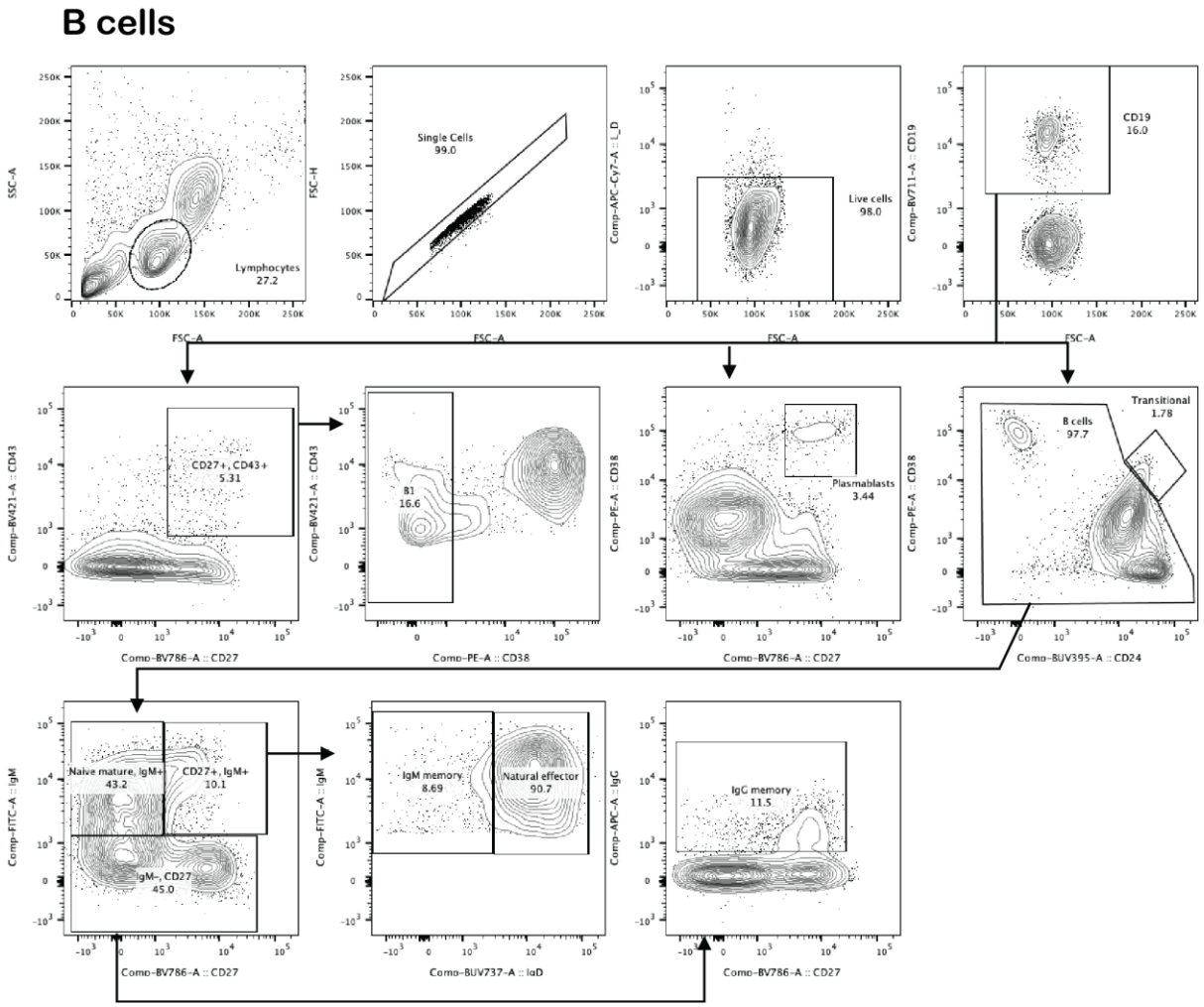


Figure-A2: Gating strategy for identifying T cell subsets



Gating strategy used to examine T cell proportions from PBMC samples by flow cytometry. A singlet gate was used to remove doublets. Cells were gated based on size (FSC vs SSC) to identify lymphocytes. CD3 was used to identify T cells (CD3+). T cells were further gated on CD4 and CD8 to identify CD4+ T cells (CD3+/CD4+) and CD8+ T cells (CD3+/CD8+). CD4+ T cells were further gated on CXCR3 and CCR6 to identify Th1 (CD4+/CXCR3+/CCR6-), Th2 (CD4+/CXCR3-/CCR6-) and Th17 (CD4+/CXCR3-/CCR6+) cells. CD4+ T cells were also gated on expression of CD45RA and CCR7 to identify CD4 effector (CD4+/CD45RA+/CCR7-), effector memory (CD4+/CD45RA-/CCR7-), central memory (CD4+/CD45RA-/CCR7+) and naïve (CD4+/CD45RA+/CCR7+) T cells .T regulatory cells (Tregs) were identified by CD127 and CD25 (CD4+/CD25+/CD127-) and further gated based on expression of CCR4 and CD45RO as naïve (CD4+/CD25+/CD127-/CCR4+/CD45RO-) and memory (CD4+/CD25+/CD127- /CCR4+/CD45RO+) Tregs. CD8 T cells were gated on expression of CD45RA and CCR7 to identify CD8 effector (CD8+/CD45RA+/CCR7-), effector memory (CD8+/CD45RA-/CCR7-), central memory (CD8+/CD45RA-/CCR7+) and naïve (CD8+/CD45RA+/CCR7+) T cells.

Figure-A3: Gating strategy for identifying B cell subsets



Gating strategy used to examine B cell proportions from PBMC samples by flow cytometry. Cells were re-gated based on size (FSC vs SSC) to identify lymphocytes. A singlet gate was used to remove doublets. A live/dead gate was used to identify live cells. CD19 was used to identify B cells (CD19+). CD19+ B cells were further gated on CD43 and CD27 and CD38 to identify B1 cells (CD19+/CD43+/CD27+/CD38-). Plasmablasts were identified by expression of CD38 and CD27 (CD19+/CD27+/CD38+) from the CD19+ gate. Further gating of CD19+ B cells using CD38 and CD24 was used to identify transitional (CD19+/CD24+/CD38+) and non-transitional cells. Non-transitional cells were gated on IgM and CD27 to identify naïve mature (IgM+/CD27-) cells. Natural effector B cells were identified from the IgM+/CD27+ gate as being IgD+ (IgM+/CD27+/IgD+). IgG memory cells were identified from the IgM- population (IgM-/CD27+/- /IgG+).

Fig.A4: Helper (CD4+) and Cytotoxic (CD8+) T cell subsets

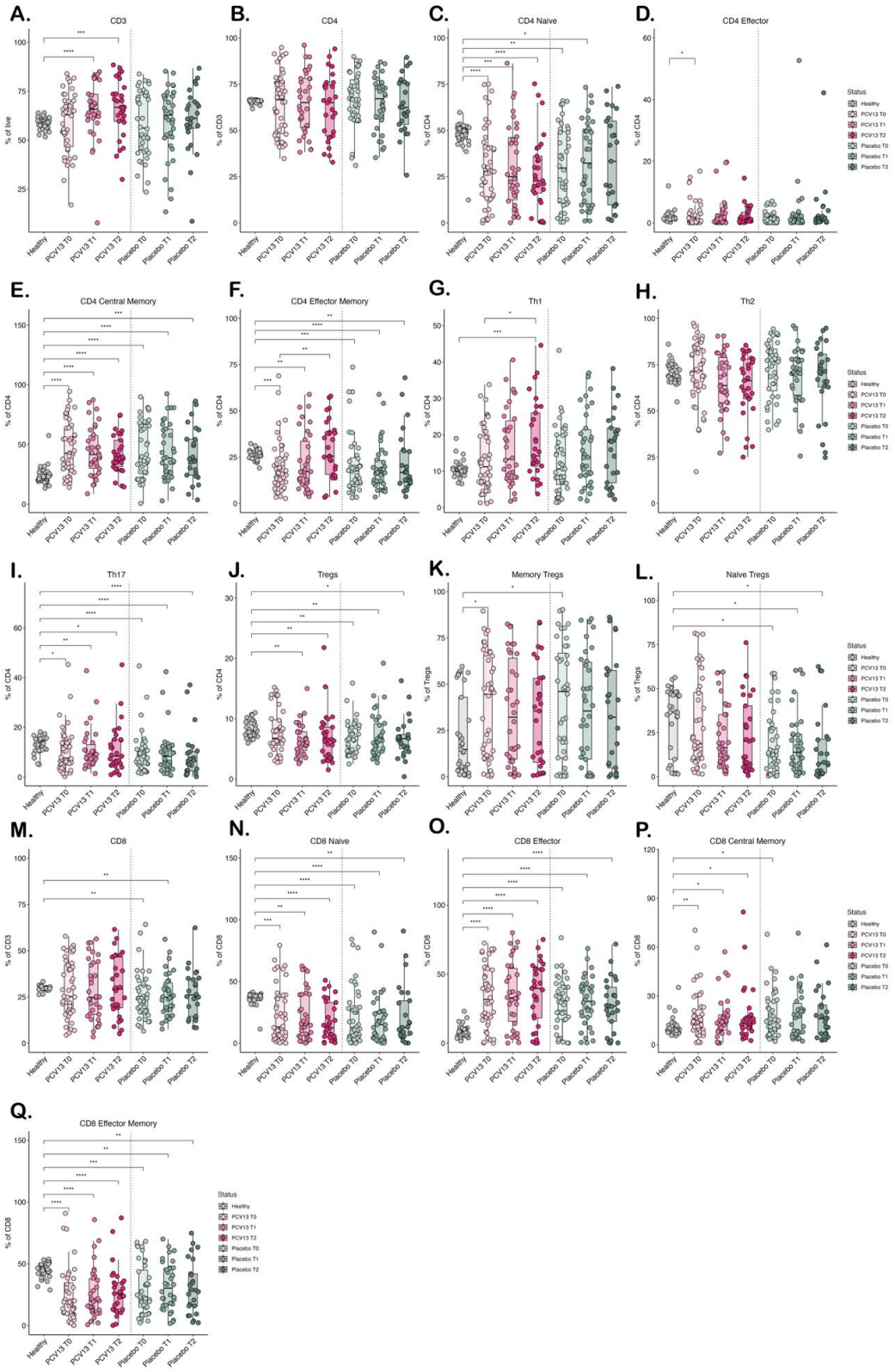
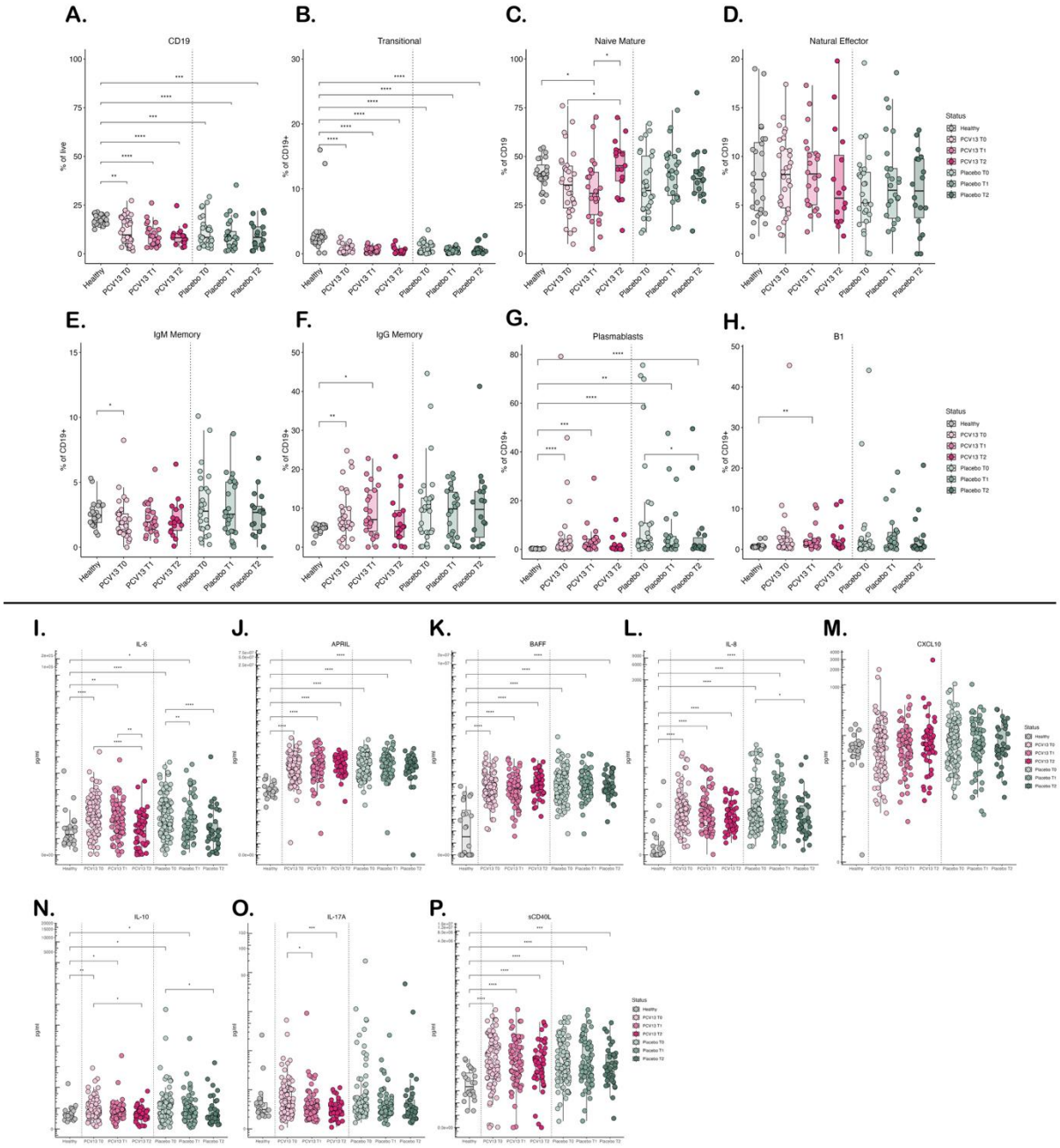


Fig.A4A: CD3+ T cells as a proportion of live cells; **Fig.A4B**: CD4 T cells(CD3+/CD4+) as a proportion of CD3+ cells; **Fig.A4C**: CD4 naive (CD4+/CCR7-/CD45RA+); **Fig.A4D**: CD4 effector (CD4+/CCR7-/CD45RA+); **Fig.A4E**: CD4 central memory (CD4+/CCR7+/CD45RA-); **Fig.S7F**: CD4 effector memory (CD4+/CCR7-/CD45RA-); **Fig.S7G**: Th1 (CD4+/CXCR3+/CCR6-); **Fig.A4H**: Th2 (CD4+/CXCR3-/CCR6-); **Fig.A4I**: Th17 (CD4+/CXCR3-/CCR6+) and **Fig.A4J**: T regulatory cells (Tregs) (CD4+/CD127- /CD25+) as a proportion of CD4 cells. **Fig.A4K**: Memory Tregs (CD4+/CD127- /CCR4+/CD45RO+) and **Fig.A4L**: naive Tregs (CD4+/CD127-/CCR4+/CD45RO-) as a proportion of Treg cells. **Fig.A4M**: Percentage of CD8 T cells as a proportion of CD3 cells; **Fig.A4N**: CD8 naive (CD8+/CD45RA+/CCR7+); **Fig.A4O**: effector (CD8+/CD45RA+/CCR7-); **Fig.A4P**: Central memory (CD8+/CD45RA-/CCR7+) and **Fig.A4Q**: Effector memory (CD8+/CD45RA-/CCR7-) lymphocyte subsets as a proportion of CD8 cells reported at T0, T1 and T2.* = p<0.05; **=p<0.01; ***p<0.001 ****p<0.0001 using parametric or non-parametric tests. For gating strategy, please see Supplementary **Fig.A2**. For reports of the median (IQR) numeric values of CD4+ and CD8+ T cell subsets see **Table 11**.

Fig.A5: B cell populations and cytokine measurements across timepoints



Proportion of B cell subsets are reported at T0, T1 and T2, with comparisons between time points with age matched healthy controls and within treatment group over-time (T1 vs T0, T2 vs T0, and T1 vs T2)). **Fig.A5A**: CD19+ B cells as a proportion of live cells; **Fig.A5B**: Transitional cells (CD19+/CD24hi/CD38+/-); **Fig.A5C**: naive mature (CD19+/CD24+/CD38+/-,CD27+/IgM+); **Fig.S8D**: natural effector (CD19+/CD24+/CD38+/-,CD27+/IgM+/IgD+); **Fig.A5E**: IgM memory (CD19+/CD24+/CD38+/-/CD27+/IgM+/IgD-); **Fig.A5F**: IgG memory (CD19+/CD24+/CD38+/- /CD27+/-/IgM-/IgG+); **Fig.S8G**: plasmablasts (CD19+/CD38+/CD27+); and **Fig.S8H**: B1 cells (CD19+/CD43+/CD27+/CD38-) as a proportion of CD19+ B cells. For gating strategy, please see Supplementary **Fig.A2**. For reports of the median (IQR) numeric values of B cell subsets see **Table 12**.

Cytokines comparisons are reported at T0 (PCV13 (n=96) vs Placebo (n=102), T1 (PCV13 (n=73) vs Placebo (n=72) and at T2 (PCV13 (n=51) vs Placebo (n=40). **Fig.A5I**: Interlukin-6; **Fig.A5J**: a Proliferation Inducing Ligand; **Fig.A5K**: B cell Activating Factor; **Fig.A5L**: Interlukin-8; **Fig.A5M**: Chemokine CXCL-10; **Fig.A5N**: Interleukin-10; **Fig.A5O**: Interleukin- 17A; **Fig.A5P**: soluble CD40 Ligand. For reports of the median (IQR) numeric values please see **Table 13** in section 19.9.3 the median (IQR) numeric values).

Fig.A6: Differentially expressed genes in the trial cohort

Fig.A6A-C: Volcano plot of panleukocyte transcriptome showing differences in gene expression between PCV13 and placebo groups at T0, T1 and T2 (P ≤ 0.01 and Log2fold change >1 or <-1).
Fig.A6D: Changes in Serotype-specific IgG by innate immune endotypes inflammation 'high' and inflammation 'low' pre-vaccination endotypes at T0 and T2. * = p<0.05; **=p<0.01; ***p<0.001, ****p<0.0001 using parametric and non-parametric tests.

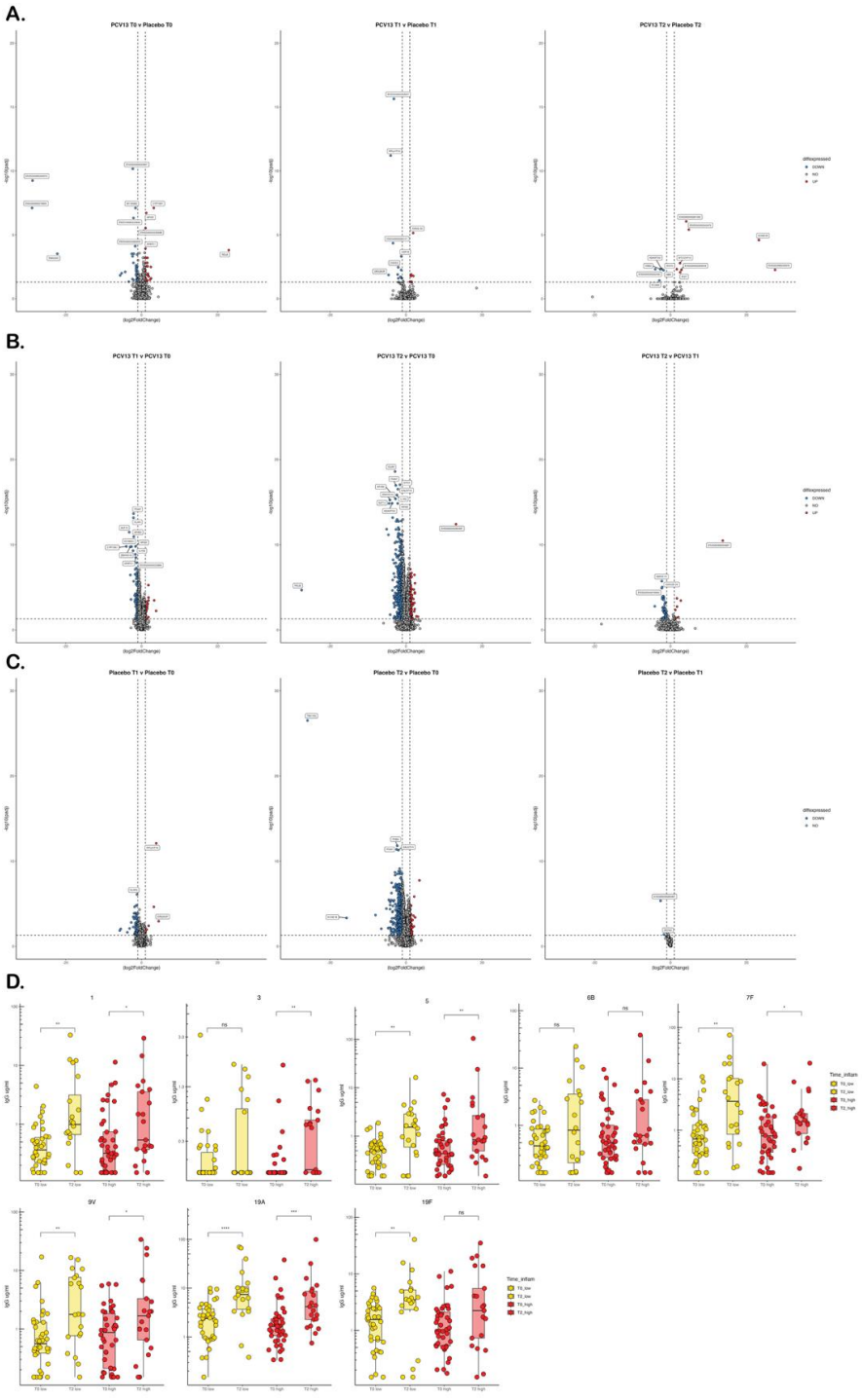
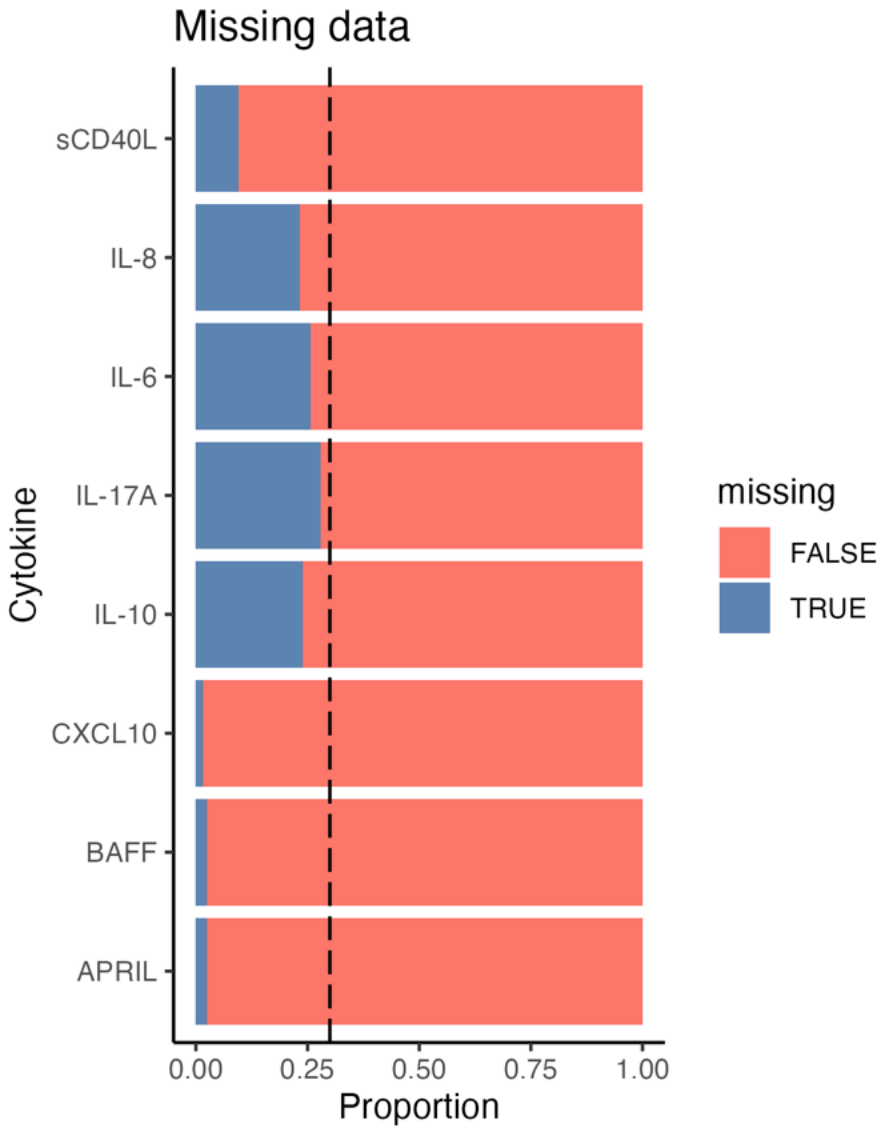


Fig.A7: Missing data in cytokine measurements

Proportion of missing data for each cytokine with limit set at 30%



Supplementary Tables

Table-S1: Characteristics of sepsis survivor population in critical care units during the VACIRiSS Trial period

Characteristic	All CMP[a] (N = 15,048)	VACIRiSS sites[b] (N = 1160)	VACIRiSS participants (N = 214)
Age (years), mean (SD)	56.0 (17.4)	54.5 (17.5)	60.2 (14.5)
Sex, n (%)			
Female	6085 (40.4)	473 (40.8)	91 (42.5)
Male	8963 (59.6)	687 (59.2)	123 (57.5)
Ethnicity, n (%)	(N = 14,296)	(N = 1043)	(N = 214)
White	12,179 (85.2)	831 (79.7)	195 (91.1)
Asian	863 (6.0)	37 (3.5)	3 (1.4)
Black	698 (4.9)	123 (11.8)	11 (5.1)
Mixed	130 (0.9)	12 (1.2)	3 (1.4)
Other	426 (3.0)	40 (3.8)	2 (0.9)
Surgical status, n (%)	(N = 15,046)	(N = 1159)	(N = 214)
Elective surgery	1261 (8.4)	98 (8.5)	27 (12.6)
Emergency surgery	4577 (30.4)	292 (25.2)	47 (22.0)
Medical	9208 (61.2)	769 (66.4)	140 (65.4)
Pre-admission dependence, n (%)	(N = 15,000)	(N = 1159)	(N = 214)
None	11,996 (80.0)	935 (80.7)	178 (83.2)
Moderate (some assistance with ADLs)	2764 (18.4)	201 (17.3)	35 (16.4)
Severe (total assistance with ADLs)	240 (1.6)	23 (2.0)	1 (0.5)
Pre-admission residence, n (%)			
Home	14,860 (98.8)	1133 (97.7)	208 (97.2)

Health-related institution	68 (0.5)	5 (0.4)	3 (1.4)
Non-health-related institution	48 (0.3)	8 (0.7)	2 (0.9)
No fixed address/abode or temporary abode	72 (0.5)	14 (1.2)	1 (0.5)
APACHE II score, mean (SD)	10.9 (4.9) [N = 14,798]	11.1 (4.8) [N = 1142]	16.7 (7.2) [N = 211]

Abbreviations: ADL, activities of daily living; APACHE II, Acute Physiology And Chronic Health Evaluation II; CMP, Case Mix Programme; SD, standard deviation.

[a] Admissions to 232 adult general critical care units in England, Wales and Northern Ireland participating in the ICNARC Case Mix Programme (CMP), during the time period of trial recruitment meeting the following criteria: age 18 years or older; sepsis identified from primary or secondary reason for admission and physiology data recorded during the first 24 hours in the critical care unit; survived to discharge from the critical care unit; and excluding pregnant admissions, admissions with APACHE II defined immune deficiency or suppression, and admissions whose prior residence was nursing home or hospice.

[b] One site in Scotland excluded as outside the geographic remit of the CMP.