PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Serotype 3 Experimental Human Pneumococcal Challenge (EHPC) study protocol; Dose ranging and reproducibility in a healthy volunteer population (Challenge 3)
AUTHORS	Hazenberg, Phoebe; Robinson, Ryan; Farrar, Madlen; Solorzano, Carla; Hyder-Wright, Angela; Liatsikos, Konstantinos; Brunning, Jaye; Fleet, Hannah; Bettam, Amy; Howard, Ashleigh; Kenny-Nyazika, Tinashe; Urban, Britta; Mitsi, Elena; El Safadi, Dima; Davies, Kelly; Lesosky, Maia; Gordon, Stephen; Ferreira, Daniela; Collins, Andrea

VERSION 1 – REVIEW

REVIEWER	Narciso, Ana Rita Karolinska Institute, Microbiology, Tumor and Cell Biology (MTC)
REVIEW RETURNED	02-Aug-2023

GENERAL COMMENTS	This study protocol involves the use of S. pneumoniae serotype 3 (SPN3) in the Experimental Human Pneumococcal Challenge (EHPC), which has been previously developed for both serotypes 6B and 15B successfully, showing safety and reproducibility. It involves the colonization with of healthy human participants with the main goal of determining optimal dose and inoculation regime, and, additionally, determining density and duration of colonization, and characterizing mucosal and systemic immune responses. This could ultimately be used for the development of new vaccines against SPN3, a serotype that still is one of the top circulating serotypes in the UK and which incidence causing IPD in all ages remained relatively unchanged after the introduction of PCV13, which includes serotype 3 capsular polysaccharide in its formulation. This protocol is clear, well formulated and detailed enough to be reproduced. Safety concerns are taken into account and all the inclusion/exclusion criteria are well defined. The amount of data collected is ambitious and could provide important information for further studies. One of the main limitations to this protocol, which the authors address, is the similarities to their previously published work (Robinson et al, AJRCCM 2022). The study design is overall very similar, with the major differences being the use of different of SPN3 strains (non-proprietary), the exclusion of the potential escalation to 160,000 CFU dose and the introduction of a reinoculation step at day 14 in case participants have tested negative after the first dose, which could potentially allow the decrease in the number of overall participants.

	Even though the study is currently ongoing and could potentially provide crucial information for further EHPC studies, I am not sure whether there is a necessity to publish a new study protocol when the parameters overall are very similar. There is also a concern that the results will eventually be too similar to the original study in terms of colonization rates and density. However, the authors do include the analysis of further immunological data which could provide key information missing from the previous study, although this is mostly post-collection analysis. Some comments: There is no mention of what strain will be used anywhere on the protocol. The previous study specified which 3 strains were used. In this case it would be interesting to know at least which clonal complex they belong to. The authors only mention the use of non-proprietary, fully sequenced and penicillin susceptible strains that "are clinically globally relevant", from the UK and Malawi. While I recognize that there is a potential for the model to be used with any strain of interest, it is relevant to know how representative this strain is and why it warranted a separate study protocol. Regarding the timeline for the participants, there is a scheduled medical screening prior to the inoculation, but there is no mention of when this visit will occur. How many days before the inoculation? This could be relevant for detection of specific exclusion criteria. There is a mention of obtaining data on long term colonization, beyond 14 days, but the latest sample is obtained at day 28 post inoculation. However, in the case of participants that will need to be reinoculated (and had no prior colonization from the first dose), and are successfully colonized post second dose, the timeline will be different. If reinoculation is now "day 0" than the latest data point will be 14 days post inoculation in these participants. The authors did not address how they will take this into account in the data analysis. They do mention that the results will be stratified but do not clarify how. In the previous SPN3 study over 30% of participants reported symptoms (in contrast to SPN6B which was reported as asymptomatic in this model). This could also affect the overall data collection and sample size. The authors use previous experience in the SPN6B and SPN3 to calculate sample size, which in this case is a minimum of 43 and a maximum of 93. They also estimate dropout/screening failure rates. But they do not address the potential for symptom development and antibiotic treatment. For example, will participants with symptoms and under antibiotic treatment still be included in the analysis and sampled at either day 2, 7 or 13? If not, will they increase the dose-ranging cohort of a specific dose? If yes, the amoxicillin treatment could potentially affect data and should be considered. Finally, the statistical analysis plan is lacking. While the analysis for the study is descriptive, if more than one dose is required (such as in the case of failure to colonize >50% of the participants at the lowest dose), it would be relevant to apply statistics on the different doses. Moreover, while the primary objective is to determine optimal dose to establish the safe colonization using the EHPC, the authors do mention exploratory goals that involve immunological studies. These are paramount to establish the baseline for the model and for future decisions regarding the right applications for the model. In this case, it would be important to

	describe which statistical analysis are involved in the immunological data analysis.

REVIEWER	Mousa, Jarrod University of Georgia
REVIEW RETURNED	17-Aug-2023

GENERAL COMMENTS	this manuscript is very good and will be helpful for the scientific community. I only have some minor questions that could be addressed: If pneumococcal vaccination has been routine since 2005 why are previously immunized patients excluded from the study? Especially in the context of serotype 3 which evades vaccine mediated antibody responses? Please provide an explanation for these and how future studies might evolve as vaccination becomes more prevalent. If a patient test positive for a viral infection, should they not be excluded? It has been well documented that viral/Spn co-infection leads to synergistic infection. How would these results be comparable to patients in the cohort without a positive viral infection? For qPCR are the primers used specific to the strains being used? How will strains be differentiated if two or more are present in any of the culturing assays?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 2

R: Many thanks for your expert review, we are glad you have enjoyed reading the study protocol.

2.1 If pneumococcal vaccination has been routine since 2005 why are previously immunized patients excluded from the study? Especially in the context of serotype 3 which evades vaccine mediated antibody responses? Please provide an explanation for these and how future studies might evolve as vaccination becomes more prevalent.

R: To ensure 'a more level immunological playing field' we have always excluded previously vaccinated participants in our human challenge (+/- vaccine) studies where we recruit 18-50 year olds. This is something that is likely to change once participants born from 2005 and after in the UK are suitable for our research studies (from age 18) - this will be the case from 2023 onwards. After this study is complete we will use the chosen SPN3 strain/dose for randomised controlled trials of new pneumococcal vaccinations. As you correctly mention we will now need to expand to include those previously PCV- vaccinated as children (noting vaccination status) - this may effect sampled size - but as you note for SPN3 it is highly likely that there is no colonisation protection so our studies will be relatively unaffected.

2.2 If a patient test positive for a viral infection, should they not be excluded? It has been well

documented that viral/Spn co-infection leads to synergistic infection. How would these results be comparable to patients in the cohort without a positive viral infection?

R: Thank you. Co-infection is indeed a hot (& very important) topic. We currently exclude participants who test positive for COVID-19 at screening/inoculation. Participants have to be well in themselves at screening and inoculation and would not be inoculated if they had any symptoms of viral respiratory tract infection within 14 days prior to inoculation. However, we acknowledge that some individuals may have asymptomatic viral infection which is why we use a viral PCR throat swab at both screening and inoculation - which is not processed in real time but later in the study. If at that stage a participant is found to be co-infected with a virus on the day of inoculation retrospectively, we will take this into account in the statistical analysis of required. Viral colonisation increases carriage rates /density but does not affect safety hence those with viral infection do not need to be excluded at the time of inoculation.

2.3 For qPCR are the primers used specific to the strains being used? How will strains be differentiated if two or more are present in any of the culturing assays?

R: Thank you for your comment. qPCR primers are specific to Serotype 3 and not exact isolate specific. We would base the qPCR result with the inoculum isolate. We can detect natural carriers using another primer/probe.

Reviewer 1:

R: Many thanks for your expert review of our study protocol.

3.1. There is no mention of what strain will be used anywhere on the protocol. The previous study specified which 3 strains were used. In this case it would be interesting to know at least which clonal complex they belong to. The authors only mention the use of non-proprietary, fully sequenced and penicillin susceptible strains that “are clinically globally relevant”, from the UK and Malawi. While I recognize that there is a potential for the model to be used with any strain of interest, it is relevant to know how representative this strain is and why it warranted a separate study protocol.

R: Thank you for asking for clarity here. The strains used are known in our laboratory as Serotype 3 10V CC700 and Serotype 3 LIV014-S3 CC180. We have now specified this in the protocol.

3.2. Regarding the timeline for the participants, there is a scheduled medical screening prior to the inoculation, but there is no mention of when this visit will occur. How many days before the inoculation? This could be relevant for detection of specific exclusion criteria.
R: Thank you. Screening occurs 5 days before inoculation (but there is a window of -7 to +4 days), we have now specified this in the protocol.

3.3. There is a mention of obtaining data on long term colonization, beyond 14 days, but the latest sample is obtained at day 28 post inoculation. However, in the case of participants that will need to be reinoculated (and had no prior colonization from the first dose), and are successfully colonized post second dose, the timeline will be different. If reinoculation is now “day 0” than the latest data point will be 14 days post inoculation in these participants. The authors did not address how they will take this into account in the data analysis. They do mention that the results will be stratified but do not clarify how.

R: Thank you for this comment and we understand you concern. We chose to not extend data collection beyond 14 days post re-inoculation as we were concerned the reimbursement for these additional visits may encourage participants to take measures to decolonise themselves after the first inoculation. The specific approach to handling individuals with multiple doses will depend on the number receiving these doses and the specific endpoint under analysis, in some cases being excluded from certain analyses. As mentioned in response to point 3.5, the full detail of the statistical analysis plan is too extensive to include in this manuscript, and as the analysis is primarily descriptive, and in many parts exploratory, we expect to adjust analyses depending on the frequency of multiple inoculations.

3.4. In the previous SPN3 study over 30% of participants reported symptoms (in contrast to SPN6B which was reported as asymptomatic in this model). This could also affect the overall data collection and sample size. The authors use previous experience in the SPN6B and SPN3 to calculate sample size, which in this case is a minimum of 43 and a maximum of 93. They also estimate dropout/screening failure rates. But they do not address the potential for symptom development and antibiotic treatment. For example, will participants with symptoms and under antibiotic treatment still be included in the analysis and sampled at either day 2, 7 or 13? If not, will they increase the dose-ranging cohort of a specific dose? If yes, the amoxicillin treatment could potentially affect data and should be considered.

R: Thank you for your comment. Whilst 30% of participants reported symptoms in the Pneumo 1 study, not all of these participants would have been given antibiotics – we use a standard operating procedure for all SPN studies which outlines specific criteria that have to be met for antibiotics to be warranted. Participants with symptoms who are given antibiotics will continue with further visits and will still be included in the analysis population as part of the intention to treat nature of the study design, but results subsequent to antibiotic treatment will be excluded (e.g. when reporting day 7 colonisation the number included will be those not treated with antibiotics prior to day 7). All attempts will be made to obtain day 2 samples prior to the participant starting antibiotics, to allow us to obtain maximal data on colonisation at day 2. We can stratify the analysis according to use of antibiotics to minimise effect on data.

3.5. Finally, the statistical analysis plan is lacking. While the analysis for the study is descriptive, if more than one dose is required (such as in the case of failure to colonize >50% of the participants at the lowest dose), it would be relevant to apply statistics on the different doses. Moreover, while the primary objective is to determine optimal dose to establish the safe colonization using the EHPC, the authors do mention exploratory goals that involve immunological studies. These are paramount to establish the baseline for the model and for future decisions regarding the right applications for the model. In this case, it would be important to describe which statistical analysis are involved in the immunological data analysis.

R: Thank you for your comment.

A detailed statistical analysis plan has been developed, but is too long to include in this protocol manuscript without considerably lengthening the manuscript. Many of the analyses are exploratory and descriptive, and an exhaustive reporting of each of these is not thought to add value to this manuscript.

If targeted booster inoculations are given, the numbers and results of these will be described, including a summary of colonisation after the second dose. Depending on the number receiving second doses, and on the endpoint being analyses, the results after the second dose will either be included as a 'new' inoculation event, potentially requiring adjustment for repeated measures, or will

be excluded. Immunological data will be described, using appropriate estimators for the characteristics of the variable (arithmetic or multiplicative means, for example) and appropriate transformations (eg. log) as required. Where appropriate, and in particular for the reproducibility cohort, regression models will be used to estimate adjusted associations between strain and number of doses with colonisation as an outcome, and with immunological response (eg IgG or antigen-specific cellular response) as an outcome.

We hope that we have sufficiently answered your questions and we look forward to your response.

Many thanks
Dr Phoebe Hazenberg, on behalf of the Challenge 3 team

VERSION 2 – REVIEW

REVIEWER	Narciso, Ana Rita Karolinska Institute, Microbiology, Tumor and Cell Biology (MTC)
REVIEW RETURNED	27-Oct-2023

GENERAL COMMENTS	The authors have addressed all my comments in a satisfactory manner.

REVIEWER	Mousa, Jarrod University of Georgia
REVIEW RETURNED	16-Oct-2023

GENERAL COMMENTS	My critiques were addressed.