PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Study Protocol for Controlled human infection for penicillin G against Streptococcus pyogenes: a double blinded, placebo controlled, randomised trial to determine the minimum concentration required to prevent experimental pharyngitis (The CHIPS trial)
AUTHORS	Hla, Thel; Osowicki, Joshua; Salman, Sam; Batty, Kevin; Marsh, Julie; Kado, Joseph; Barr, Renae; Enkel, Stephanie L.; Snelling, Thomas; McCarthy, James; Steer, Andrew C.; Carapetis, Jonathan; Manning, Laurens

VERSION 1 – REVIEW

REVIEWER	Malick Gibani Imperial College London
REVIEW RETURNED	10-Aug-2022

GENERAL COMMENTS	Thel Hal and colleagues describe a study protocol for the CHIPS trial - a randomized, double-blinded, placebo-controlled human infection study of Streptococcus pyogenes. This study builds upon the experience of this group in delivering Streptococcal challenge as described in the CHIVAS-M75 study. Overall, this is a well-written, clear, and appropriately detailed protocol paper. The rationale for the study is appropriately discussed in the introduction, including barriers to adherence to penicillin prophylaxis. It represents a novel application of the group A Strep human challenge model. The study aims to determine the minimum plasma penicillin concentration required to protect against S. Pyogenes pharyngitis, within the context of a controlled human infection model. The study team describes the composite definition of pharyngitis using clinical scoring, examination of tonsillar size, and point-of-care diagnostics. Prevention of colonization is defined as a secondary endpoint, alongside other pharmacological and immunological measurements. Figure 1 clearly describes the participant journey well and Figure 3 nicely illustrates the relationship between the trial management group and the safety data review team. Prior to the challenge, participants will be administered a bolus dose of penicillin and have serial blood samples collected. The study team describe how they will measure clearance and volume of distribution to calculate an individualized dosing regimen required to achieve a target concentration. Intravenous infusions will commence prior to the challenge to attain a steady state at one of five concentrations (including placebo). Participants will typically be challenged in cohorts of 5. Antibiotics (azithromycin) will be initiated at the end of the quarantine period or when they develop pharyngitis.

	The discussion section nicely describes the potential future use cases and limitations of the mode. Overall, I have no major critiques of this paper as written. It will be a valuable resource for other researchers conducting controlled human infection studies.

REVIEWER	Thea Brennan-Krohn Boston Children's Hospital
REVIEW RETURNED	13-Sep-2022

GENERAL COMMENTS	General comments: The authors present a protocol for an innovative controlled human infection study designed to determine the minimum penicillin concentration required to prevent acquisition of group A streptococcal pharyngitis. This is an important first step toward developing more practical and patient-friendly approaches to secondary prophylaxis for acute rheumatic fever. The protocol for the primary objective is clearly described and the article is well-written. However, the authors include quite a few secondary outcome measures in Table 1, yet apart from secondary outcomes 9 (environmental contamination) and 10 (participant experiences), there is no information anywhere in the manuscript about these aims. It isn't clear to me why procedures for these two outcomes are described in detail but the others are not mentioned at all. I think the authors need to provide some clarification on these, and if some of these are proposed outcomes for which no specific planning has yet been carried out then this should be specified. Several of the outcomes involve specific interventions on study subjects that presumably had to be approved by the ethics committee (e.g. blood draws for numerous proposed laboratory assays, saliva collection for saliva penicillin concentrations determination, and "laboratory assays to measure mucosal response"), so I would assume that they have determined how these would be carried out. Specific suggestions: Lines 58-69: Only strengths are listed in "Strengths and Limitations"; I would suggest also including the limitations that the authors discuss in the Discussion section. Line 76: Minor point, but would reword "3- to 4-weekly intramuscular (IM) injections" to clarify that this means injections every 3 to 4 weeks. Lines 182-183 and lines 245-246: Is there evidence to support the use of azithromycin to reduce the risk of long-term carriage in people with acute streptococcal pharyngitis? I know there is data on the efficacy of azithromycin in eradicating established GAS carriage, but I'm not aware of any data showing that using a different agent for initial treatment of streptococcal pharyngitis prevents carriage. Even if such data exists, I think it would be worth explaining in more detail the decision to use azithromycin rather than penicillin as the treatment regimen, since penicillin is the only antibiotic that has specifically been demonstrated to prevent acute rheumatic fever, which it seems would be an important ethical priority for volunteers being infected with S. pyogenes. I'm assuming that the thought is that for patients randomized to penicillin who develop infection during the infusion there might be some kind of in vivo penicillin tolerance, but presumably in most cases infection would instead be due to inadequate penicillin concentrations, and I would still think

	penicillin would be the first-line treatment in those cases (and certainly for people in the placebo arm). Was there consideration of using penicillin as initial treatment, followed by delayed screening for carriage and treatment of carriers at that point with an agent like azithromycin? Lines 238-239: Is there any systematic plan for monitoring for secondary cases of S. pyogenes in non-participants? Table 1: Secondary Objective 7 appears to be a duplicate of Secondary Objective 6. Table 1: For Secondary Objective 8 ("To explore S. pyogenes transcriptomic changes in response to penicillin exposure in S. pyogenes pharyngitis"), the Endpoint/Outcome does not appear correct ("Laboratory assays to measure mucosal response"). Supplementary Material: Inclusion Criteria, item 4: Suggest re-phrasing "non-clinically significant laboratory profiles" – I assumed this means something like "laboratory profiles without any clinically significant abnormalities" or something to this effect. Exclusion Criteria, item 2: Why is use of NSAIDs excluded? This seems like an exclusion criteria that warrants justification because some of the people in this study will develop pharyngitis as a result of the study intervention and will want to use analgesics. Exclusion Criteria, item 12: Why are COVID and influenza vaccines exempted from the vaccine exclusion criteria? I'm assuming this is primarily a practical decision, as many potential participants will likely have recently received these vaccines, but if there is a concern that recent vaccination will affect immune responses (which I'm assuming is the reason for the general vaccine exclusion criteria), I would think these vaccines would have similar issues.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 Comments:

1. **Dr. Malick Gibani, Imperial College London Comments to the Author:**

   Response: We thank and appreciate the positive review from Dr Gibani.

Reviewer 2 Comments:

Dr. Thea Brennan-Krohn, Boston Children's Hospital Comments to the Author:

2. **However, the authors include quite a few secondary outcome measures in Table 1, yet apart from secondary outcomes 9 (environmental contamination) and 10 (participant experiences), there is no information anywhere in the manuscript about these aims. It isn't**

clear to me why procedures for these two outcomes are described in detail but the others are not mentioned at all. I think the authors need to provide some clarification on these, and if some of these are proposed outcomes for which no specific planning has yet been carried out then this should be specified. Several of the outcomes involve specific interventions on study subjects that presumably had to be approved by the ethics committee (e.g. blood draws for numerous proposed laboratory assays, saliva collection for saliva penicillin concentrations determination, and "laboratory assays to measure mucosal response"), so I would assume that they have determined how these would be carried out.

Response: Please also see the response to the Editor (comment no. 2) above. As the reviewer has insightfully pointed out, some of the proposed outcomes are exploratory and therefore, specific plans are not yet in place for their analysis and reporting. We can confirm that HREC approval has been obtained for collection of all samples described and that seperate and optional informed consent will be sought from for use of these samples in future studies.

3. **Lines 58-69: Only strengths are listed in "Strengths and Limitations"; I would suggest also including the limitations that the authors discuss in the Discussion section.**

   Response: Thank you. We have revised these as requested by the editor and reviewer.

4. **Line 76: Minor point, but would reword "3- to 4-weekly intramuscular (IM) injections" to clarify that this means injections every 3 to 4 weeks.**

   Response: Wording now changed as recommended.

5. **Lines 182-183 and lines 245-246: Is there evidence to support the use of azithromycin to reduce the risk of long-term carriage in people with acute streptococcal pharyngitis? I know there is data on the efficacy of azithromycin in eradicating established GAS carriage, but I'm not aware of any data showing that using a different agent for initial treatment of streptococcal pharyngitis prevents carriage. Even if such data exists, I think it would be worth explaining in more detail the decision to use azithromycin rather than penicillin as the treatment regimen, since penicillin is the only antibiotic that has specifically been demonstrated to prevent acute rheumatic fever, which it seems would be an important ethical priority for volunteers being infected with S. pyogenes. I'm assuming that the thought is that for patients randomized to penicillin who develop infection during the infusion there might be some kind of in vivo penicillin tolerance, but presumably in most cases infection would instead be due to inadequate penicillin concentrations, and I would still think penicillin would be the first-line treatment in those cases (and certainly for people in the placebo arm). Was there consideration of using penicillin as initial treatment, followed by delayed screening for carriage and treatment of carriers at that point with an agent like azithromycin?**

   Response: Although treatment recommendations for acute streptococcal pharyngitis generally recommend penicillin (or amoxicillin) in preference to alternative drugs, and it remains unclear if there are clinically relevant differences between different antibiotics for this indication (PMID: 33728634), microbiological treatment failure with penicillin treatment of acute pharyngitis is well described (PMID: 18036409, 11694700). There are also data showing more frequent microbiological eradication with macrolides (including higher-dose shorter-duration azithromycin) and cephalosporins (and even amoxicillin) compared to phenoxymethylpenicillin and benzathine penicillin (e.g. PMIDs 15156437; 15909262; 16767482; 17908614; 16199251; 19285734; 14770076; 12739920).

   For this study, azithromycin was selected as the preferred antibiotic for all participants, regardless of whether the primary endpoint was met. The choice was a pragmatic one, based on a number of factors:

   i) Azithromycin is effective for the treatment of streptococcal pharyngitis
   ii) Azithromycin is a recommended second-line therapy in Australian clinical guidelines.

iii) Although we believe that clinically meaningful penicillin tolerance is unlikely, we were mindful of this possibility in those exposed to subtherapeutic concentrations of penicillin. We agree that the likely mechanism of breakthrough pharyngitis relates to inadequate penicillin concentrations, just as it does for breakthrough pharyngitis in ARF patients who receive regular intramuscular benzathine penicillin prophylaxis. Still, local ARF/RHD guidelines have typically suggested an alternative antibiotic besides penicillin is used in that scenario of breakthrough pharyngitis.

~~iv)~~ The goal in the human challenge trial setting has been to select a simple treatment regimen associated with high microbiological eradication and adherence (shorter course, fewer doses). In the initial CHIVAS-M75 trial, a single dose of intramuscular benzathine penicillin and 4 days of rifampicin two-times-a-day (rifampin) was used, drawing on the 'carriage' literature and maximising adherence by delivering the injection and the first day of rifampicin while the participants were still inpatients. There were zero microbiological failures. Having undertaken that first trial, the preference of the investigators was to avoid further intramuscular antibiotic treatment. Higher-dose shorter-course azithromycin meets many of the requirements. Also, our preference is to apply the same antibiotic treatment across all participants, to maintain blinding and reduce variability that may affect subsequent bacteriological and immunological investigations.

Finally, we are aware that penicillin is the only antibiotic with proven efficacy in preventing ARF in patients at risk of RHD. However, CHIPS participants will be screened to be at the lowest possible risk of RHD. We have previously addressed the risk of rheumatic fever in this model (PMID: 31101422). It is worth mentioning that for those healthy adults eligible to participate in this challenge trial, sore throat guidelines in Australia, New Zealand, and the UK would not recommend either testing or treating (different from US guidelines). First episode acute rheumatic fever in healthy adults in these settings is exceedingly rare and essentially unheard of now (just as it is in the US) and would be expected to be rarer still with early antibiotic therapy active against the infecting strain.

6. **Lines 238-239: Is there any systematic plan for monitoring for secondary cases of *S. pyogenes* in non-participants?**

   Response: Monitoring of secondary cases will be via self-reporting. During the period of confinement following challenge, only clinical staff using recommended infection control precautions will have contact with the participants. The risk of transmission in this setting is extremely low. By the time of discharge, participants will have received 2 doses of oral azithromycin, reducing the risk of onwards transmission to household and other contacts markedly, as is recognised in recommendations across many countries for exclusion of pharyngitis cases from school for 24 hours after antibiotic treatment (12-24 hours in the USA, 24 hours in Australia and the UK). These recommendations are based on very high rates of early microbiological eradication across many previous clinical trials and reflected in results from the CHIVAS-M75 study.

   In the event that a secondary transmission is suspected, this will be investigated as a medically significant adverse event, with relevant clinical records and pathology results reviewed (with consent from secondary contacts).

7. **Table 1: Secondary Objective 7 appears to be a duplicate of Secondary Objective 6.**

   Response: Thank you for noting this error. Duplicate now removed.

8. **Table 1: For Secondary Objective 8 ("To explore S. pyogenes transcriptomic changes in response to penicillin exposure in S. pyogenes pharyngitis"), the Endpoint/Outcome does not appear correct ("Laboratory assays to measure mucosal response").**

   Response: The endpoint/ outcome now edited to better reflect the objective.

Supplementary Material:

9. **Inclusion Criteria, item 4: Suggest re-phrasing "non-clinically significant laboratory profiles" – I assumed this means something like "laboratory profiles without any clinically significant abnormalities" or something to this effect.**

   Response: The wording now changed as suggested.

10. **Exclusion Criteria, item 2: Why is use of NSAIDs excluded? This seems like an exclusion criteria that warrants justification because some of the people in this study will develop pharyngitis as a result of the study intervention and will want to use analgesics.**

    Response: This restriction is in place to address the possibility of NSAIDs' association with severe *S. pyogenes* infection. An association between use of NSAIDs and invasive *S. pyogenes* infection is well described in the literature, especially with recency of use (PMID: 8645850, 12861100, 12967496). Although a clear causal association is not established, physiological plausibility has been suggested and there is data from animal studies (PMID: 21697021). Therefore, NSAIDs were excluded as a safety consideration for our participants. It is also plausible that NSAIDs may meaningfully confound the results of mucosal and systemic immunology assessments. As in CHIVAS-M75, participants will be offered analgesia with paracetamol (acetaminophen) at standard doses, with low-dose opioids reserved for severe pain (none were administered in CHIVAS-M75).

11. **Exclusion Criteria, item 12: Why are COVID and influenza vaccines exempted from the vaccine exclusion criteria? I'm assuming this is primarily a practical decision, as many potential participants will likely have recently received these vaccines, but if there is a concern that recent vaccination will affect immune responses (which I'm assuming is the reason for the general vaccine exclusion criteria), I would think these vaccines would have similar issues.**

    Response: As suggested by the reviewer, this was predominantly a practical decision reflecting the reality of recruiting healthy volunteers in the midst of the COVID-19 pandemic. COVID-19 vaccination remains mandatory in Australia in some settings, and strongly recommended for all other adults. Similarly, influenza vaccination is recommended in Australia during the influenza season for most adults. Having said that, circumstances have changed somewhat since the time when this protocol was initially prepared, and it is now less likely that potential participants will have recently been vaccinated with a COVID-19 vaccine.

VERSION 2 – REVIEW

REVIEWER	Thea Brennan-Krohn Boston Children's Hospital
REVIEW RETURNED	29-Sep-2022

GENERAL COMMENTS	The authors have provided comprehensive and thoughtful responses to my suggestions and questions.