PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Chlorhexidine oral rinses for symptomatic COPD: a randomized, blind, placebo-controlled preliminary study
AUTHORS	Pragman, Alexa; Fieberg, Ann; Reilly, Cavan; Wendt, Christine

VERSION 1 – REVIEW

REVIEWER	Mina, Bushra Lenox Hill Hospital, Pulmonary critical care medicine
REVIEW RETURNED	12-May-2021

GENERAL COMMENTS	The article has clinical significance as it addresses one of the questions regarding etiology of acute exacerbation of COPD which could be related to bacterial infection in #50% of the time and whether oral bacteria has an impact on it. In my opinion, the controlled randomized design added to the strength of the study. Comments: The number of participant is low which could have impacted on the statistical analysis. No diversity, most participant were white males Did not standardize daily personal daily dental care habits Did not address in details the discrepancy between changes in the daily respiratory symptoms and improvement in quality of life on the other hand the limitation of the study were illustrated

REVIEWER	Boisson, Matthieu Centre Hospitalier Universitaire de Poitiers, Anaesthesia and Intensive Care Unit
REVIEW RETURNED	12-Jun-2021

GENERAL COMMENTS	Review for the manuscript “Chlorhexidine oral rinses for symptomatic COPD: a randomized, blind, placebo-controlled preliminary study” by Pragman AA et al. This manuscript the result of monocentric, randomized, blind, placebo-controlled preliminary study of the effect of Chlorhexidine oral rinses for symptomatic COPD. Forty four participants were randomized 1:1 to twice-daily 0.12% chlorhexidine oral rinses versus placebo for 2 months along with daily diaries. The primary outcome was a change in oral and sputum microbiota biomass compared to baseline assessed by 16S rRNA quantification. Secondary outcomes were changes in oral and sputum microbiota Shannon and Simpson diversity, taxonomy, inflammations assed by WBC, fibrinogen and CRP and quality scores (BCSS, SGRQ). Major results were decreased in oral and sputum microbiota alpha diversity and in clinical symptoms (SGRQ) in the Chlorhexidine group. Primary outcome did not reach significancy. The study

	respected the ethical rule, was declared at the beginning of the enrollment. The manuscript respected the consort Guidelines. The main strengths of this study are its originality, its design, its quite long follow-up for such intervention. The major limitations of the study are the sample size calculations based on a secondary endpoint and the non-exclusion of patients treated with antibiotics during the study. So some issues should be addressed by the authors. Major comments 1- Even if for a preliminary study, it is unusual to determine the number of subjects that should be included with a secondary endpoint. This could be explain the negative result of the study particularly in sputum and should be clearly appear as the limit of study in the discussion. 2- Although patients with previous antibiotic treatment were excluded, patients treated with antibiotics during the study were retained in the analyses, a sub-group analysis was performed for biomass but not for diversity analysis. I suggest to the authors to provide the results of the diversity analysis for the sub-group without antibiotic in the main manuscript. 3- The author chose to normalize their oral and sputum samples to their samples mass. But data were missing for one third of them which is quite huge! Why did they not normalize by the number of cells? 4- In the discussion, the authors should discuss their results with literature. There is no reference citation in the manuscript and it is not clear for reviewers if the statements were support by the literature. Minor comments 1- Apart from the phone call at Week 4, how was the respect of intervention controlled? 2- In my opinion, the Results part is a bit confusing and should be simplified. Some sentences should be in the methods part and another ones in the discussion. 3- For diversity analysis, authors should present the metrics validating the quality of their run (i.e rarefaction curve) 4- Tables 3a and 3b could be simplified and merged 5- In Table 5, inflammatory biomarkers could be removed and presented by a simple sentence in the results part 6- Table 6 could be removed and presented by a simple sentence in the results part

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Bushra Mina, Lenox Hill Hospital

1. The number of participants is low which could have impacted on the statistical analysis.

Response: Our preliminary study of 44 patients was not powered to definitively reject all the null hypotheses addressed in our study. Nevertheless, we were able to identify several positive findings (alpha diversity dysbiosis, SGRQ score changes) which warrant follow up in a larger study. We have carefully presented our results as preliminary findings. As such, our negative results may be impacted by low statistical power and a relationship cannot be definitively ruled out at this time. We have included a statement summarizing this concern at the end of the first paragraph of the Discussion section.

2. No diversity, most participant were white males.

Response: Our single-center study was performed at a Department of Veterans Affairs medical center and we were limited to recruiting from our current patient population. We agree that a larger study, recruiting from a more diverse patient population across multiple institutions, will offer more conclusive and generalizable results in the future. A statement regarding our homogeneous patient population is now included among the study limitations in the Discussion section.

3. Did not standardize daily personal daily dental care habits.
Response: Our objective was to determine the effect of twice-daily chlorhexidine oral rinses, not the effects of multiple oral care practices. As this is a randomized study, it is unlikely that participants' various other oral care practices were unequally distributed between the two study groups. The randomization process makes it unlikely that participants' other oral care practices confounded our analyses.

4. Did not address in detail the discrepancy between changes in the daily respiratory symptoms and improvement in quality of life.
Response: The BCSS strictly evaluates the severity of respiratory symptoms on a daily basis. In contrast, the SGRQ more broadly assesses the impact of these symptoms on an individual's activities and quality of life, and asks participants to reflect more broadly on symptoms over the prior month. These nuances are discussed in the manuscript Discussion section (3rd-to-last paragraph). Additionally, when we inspected the BCSS scores and week-by-week changes in BCSS scores by treatment group, we discovered that while those using chlorhexidine did see an improvement in BCSS score throughout the 8-week study, those using placebo also saw an improvement in BCSS score as well. The placebo group's BCSS score improvement appears to have happened later in the study and may have been less consistent than the improvement seen among the chlorhexidine group. We chose not to include these observations in the manuscript as we lacked statistical power to evaluate them. It remains plausible that a larger study performed over a longer period of time may identify an improvement in both the BCSS and SGRQ scores.

Reviewer: 2
Dr. Matthieu Boisson, Centre Hospitalier Universitaire de Poitiers, Universite de Poitiers UFR Medecine et Pharmacie

Major comments
1. Even if for a preliminary study, it is unusual to determine the number of subjects that should be included with a secondary endpoint. This could explain the negative result of the study particularly in sputum and should be clearly appear as the limit of study in the discussion.
Response: We agree that studies are typically powered to address the primary endpoint. However, when we started this study, no data on lung microbiome 16S copy number changes due to chlorhexidine use were available. We therefore chose to use alpha diversity changes in the lung microbiome (specifically, bronchoalveolar lavage fluid) for our power calculations instead. We chose to use alpha diversity in bronchoalveolar lavage because it is a very conservative estimate of the power needed to address 16S copy numbers in oral wash or sputum, the primary endpoint. Certainly, an antiseptic like chlorhexidine (which is broadly active against both Gram positive and Gram negative bacteria) is expected to decrease the number of bacteria more readily than it would change the biodiversity of the remaining bacteria. Furthermore, any changes detected in the low biomass bronchoalveolar lavage fluid would be much more notable in the higher biomasses seen in the oral and sputum microbiota. In this context, we do not believe that our choice to power the study for a secondary endpoint is responsible for not detecting our primary outcome. We have now included the use of biodiversity in our power calculations as a limitation of the study (Discussion section, 3rd from last paragraph).

2. Although patients with previous antibiotic treatment were excluded, patients treated with antibiotics

during the study were retained in the analyses, a sub-group analysis was performed for biomass but not for diversity analysis. I suggest to the authors to provide the results of the diversity analysis for the sub-group without antibiotic.
Response: Our manuscript primarily utilizes an intention-to-treat analysis as this is a more rigorous evaluation of the intervention than a per-protocol analysis. At the reviewer's request, we now include the sub-group analysis of biodiversity of those who did not receive an antibiotic in Table S2. This subgroup exhibited remarkably similar decreases (which remained statistically significant) in both oral wash and sputum sample biodiversity indices as the result of chlorhexidine use. This demonstrates that nearly identical effects of chlorhexidine use on biodiversity are detected regardless of antibiotic use during the study period.

3. The author chose to normalize their oral and sputum samples to their samples mass. Why did they not normalize by the number of cells?
Response: When evaluating sputum findings, we wanted to ensure that we were normalizing our results to the amount of sample each subject produced (which is effort-dependent), rather than the amount of inflammation in the sample (which may have been influenced by treatment allocation and which could have confounded the results). Therefore, we normalized sample findings to sputum weight (the amount of each sample) rather than the number of cells in the sample (how inflammatory the sample was). Normalizing our findings as suggested by the reviewer would not have addressed our outcome of interest: how many bacterial 16S rRNA copies are present in a sample when we control for the amount of sample the subject provided?

4. In the discussion, the authors should discuss their results with literature. There is no reference citation in the manuscript and it is not clear for reviewers if the statements were support by the literature.
Response: We thank the reviewer for pointing out that the literature cited in the introduction was not again referenced in the discussion section. We have now inserted the relevant citations in the discussion section and included two additional manuscripts related to lung microbiome alpha diversity and COPD biomarkers (references 20 and 21).

Minor comments
1. Apart from the phone call at Week 4, how was the respect of intervention controlled?
Response: In addition to calling subjects at week 4 to reinforce study drug adherence and completion of the BCSS daily diaries, we also asked subjects to return their empty study drug bottles at the end of the study. At the week 8 visit, the coordinator made note of any remaining study drug (a statement to this effect has been added to the supplementary methods). There is no readily available, objective method to confirm regular use of oral chlorhexidine. We did not intend to perform a per-protocol analysis, so information on study drug compliance was not analyzed.

2. In my opinion, the Results part is a bit confusing and should be simplified. Some sentences should be in the methods part and another ones in the discussion.
Response: We understand the reviewer's concern about manuscript readability and complexity. We have simplified the Results section and taken opportunities to move some information to the Methods (or supplemental methods) and Discussion.

3. For diversity analysis, authors should present the metrics validating the quality of their run (i.e rarefaction curve).
Response: We understand the reviewer's concerns, and in our previous publications on the lung microbiome we included additional technical details regarding the sequencing run. By necessity, in presenting these clinical trial data, we had to prioritize adherence to the CONSORT guidelines. To address readers' concerns about the quality of our sequencing run, we have included our rarefaction curve as Figure S1 in the supplemental methods as requested.

4. Tables 3a and 3b could be simplified.
Response: We thank the reviewer for this excellent suggestion to simplify the manuscript. Table 3a is now only in the supplement (now Table S1) and the former Table 3b is now Table 3 in the main manuscript.

5. In Table 5, inflammatory biomarkers could be removed and presented by a simple sentence in the results part.
Response: We thank the reviewer for this excellent suggestion as well. Table 5 has been simplified in the main manuscript by removing the data on the inflammatory markers. The information on the inflammatory biomarkers is now available in the supplement and the results are summarized in the text.

6. Table 6 could be removed and presented by a simple sentence in the results part.
Response: We thank the reviewer for this suggestion as well, and have decided to remove Table 6 from the manuscript for simplicity. However, this information is easier to summarize in a tabular format, so we have moved the table to the supplement (Table S4).

Recruitment timing in relationship to the finalization of the ClinicalTrials.gov submission should be clarified.
Response: Submission of the trial to ClinicalTrial.gov was coordinated through the University of Minnesota Clinical and Translational Science Institute. Unfortunately, there was a misunderstanding regarding the submission completion date and the first patient was inadvertently randomized prior to completing entry into ClinicalTrials.gov. After this was discovered, the study was paused until full submission was completed.