PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Stratified Assessment of the Role of Inhaled Hypertonic Saline in Reducing Cystic Fibrosis Pulmonary Exacerbations
AUTHORS	Dmello, Dayton; Nayak, Ravi; Matuschak, George

VERSION 1 - REVIEW

REVIEWER	***Scott H. Donaldson, MD*** Associate Professor of Medicine University of North Carolina at Chapel Hill No competing interests
REVIEW RETURNED	19-Nov-2010

THE STUDY	1. Patient population: there were 340 exacerbations - don't describe how many patients they occurred in (would guess 424 from Table 1) 2. Did any patients switch between HS using/non-use groups during the 3 year period? How was this handled? 3. Apparently the frequency of exacerbations is being compared in the various groups - the actual frequencies are not reported (only odds ratios). It is also not clear to me whether they are comparing proportion of patients in HS using/nonusing groups who had any exacerbation during 3 years, or the frequency of exacerbation in each subject. Since a Chi squire analysis was used, presume the former; but their text says they are comparing the frequency (a continuous variable). 4. Many details regarding analysis and the population are missing 5. They show multiple baseline differences between the using/non-using populations, including many that would likely impact exacerbation frequency (gender, BMI, baseline FEV1, pseudomonas positivity, MRSA positivity), yet make no attempt to adjust for these variables.
RESULTS & CONCLUSIONS	Don't have confidence in the data analysis, as described above, and lack information regarding the patients and their exacerbation frequencies.

REVIEWER	***Peter Wark*** Department of Respiratory and Sleep Medicine John Hunter Hospital New Lambton
REVIEW RETURNED	24-Nov-2010

THE STUDY	Given this is a retrospective Cf study a think a fuller description of the subjects should be provided. In the table should include, use of airway clearance techniques, use of regular antibiotics, presence of other co-morbuidities such as diabetes etc. It is unclear to me why a regression analysis could not be done,

	recommend statistical opinion in regard to this. If so are alternatives available to use in the context of a retrospective observational design?
RESULTS & CONCLUSIONS	I think a greater description of the subjects is needed to formulate this opinion to determine other factors that are known to be associated with exacerbation frequency.
GENERAL COMMENTS	I acknowledge the limitations that any retrospective review of databases will have and these are addressed by the authors. Nonetheless the use of data from these databases is invaluable to provide insight that does not currently exist. I probably am supportive of this endeavour. However in the current form there is not an adequate description of subjects. It is also unclear why only those with exacerbations were chosen for analysis, why not all subjects on the database? As it stands this only addresses the application in those who do have exacerbations. Further analysis to assess confounding of multiple factors should still be possible, I would be guided by statistical review here but certainly pose the question prior to publication.

VERSION 1 – AUTHOR RESPONSE

We wish to sincerely thank the reviewers for the time and effort taken to review this manuscript. Due to the strong recommendations from both reviewers to include a multivariate analysis in the study, we have performed a subgroup logistic regression analysis that has significantly strengthened our study findings, and has in fact led to a revised conclusion. In this context, this particular suggestion has been invaluable and the authors convey their special thanks. Also, we have listed the regression tables in the Appendix but defer to both reviewers to decide if they need inclusion in the actual manuscript.
Finally, we appreciate the opportunity to address issues and concerns raised in a point-by-point manner below and where indicated in the revised paper. All changes in the revised manuscript have been highlighted in yellow text, as well as listed by numerical page and line number below.

Reviewer 1

Comment 1
Patient population: there were 340 exacerbations - don't describe how many patients they occured in (would guess 424 from Table 1)
Response 1:
We thank the reviewer for requesting this clarification. The reviewer is correct, the total is 424 patients as depicted in Table 1.

Comment 2
Did any patients switch between HS using/non-use groups during the 3 year period? How was this handled?
Response 2
We thank the reviewer for requesting this clarification. Our database records the use of inhaled HS at every visit, and also at the time of each exacerbation. To the best of our knowledge, there were no cases that had prolonged periods of switching between HS/non-HS use; however, we acknowledge that there was no methodology to assess ongoing compliance, and this may have been a confounding factor.
•See Page 11, Lines 5-8

Comment 3

Apparently the frequency of exacerbations is being compared in the various groups - the actual frequencies are not reported (only odds ratios). It is also not clear to me whether they are comparing proportion of patients in HS using/nonusing groups who had any exacerbation during 3 years, or the frequency of exacerbation in each subject. Since a Chi squire analysis was used, presume the former; but their text says they are comparing the frequency (a continuous variable).
Response 3
We thank the reviewer for requesting this important clarification.
We do clarify that the reviewer is correct, we are comparing the proportion of patients in HS/non-HS groups with exacerbations over a 3-yr period. We have amended our text to remove the word "frequency".
•See Page 2, Line 15

Comment 4
Many details regarding analysis and the population are missing
Response 4
We thank the reviewer for this comment.
We have already listed population characteristics in Table 1 showing the demographical characteristics such as Age, BMI and Gender, as well as the clinical variables of FEV1, FVC, sputum positivity for Pseudomonas and MRSA, and rhDNase use. We have now added the additional variables of mechanical airway clearance device usage as well as the proportion of patients necessitating hospitalization versus home intravenous antibiotic administration.
•See Page 8, Table 1, Lines 40-48
As far as the analysis goes, we have listed the univariate analysis findings in Table 2 with the new multivariate analysis results by logistic regression in Table 3. Detailed regression analyses tables are provided in the Appendix.
•See Page 9, Tables 2 & 3, Lines 3-41
•See Supplemental tables in Appendix

Comment 5
They show multiple baseline differences between the using/non-using populations, including many that would likely impact exacerbation frequency (gender, BMI, baseline FEV1, pseudomonas positivity, MRSA positivity), yet make no attempt to adjust for these variables
Response 5
We acknowledge that this was a major design flaw. Accordingly we have performed a regression analysis at each level of severity and have included our results in the revised manuscript.
•See Page 6, Lines 25-30
•See Page 7, Lines 3-16
•See Page 9, Table 3, Lines 24-41
•See Supplemental tables in Appendix

Reviewer 2

Comment 1
Given this is a retrospective Cf study a think a fuller description of the subjects should be provided. In the table should include, use of airway clearance techniques, use of regular antibitoitcs, presence of other co-morbidities such as diabetes etc.
Response 1
We thank the reviewer for requesting this clarification. We have already listed population characteristics in Table 1 showing the demographical characteristics such as Age, BMI and Gender, as well as the clinical variables of FEV1, FVC, sputum positivity for Pseudomonas and MRSA, and rhDNase use. We have now added the additional variables of mechanical airway clearance device

usage as well as the proportion of patients necessitating hospitalization versus home intravenous antibiotic administration.
•See Page 8, Table 1, Lines 40-48
All patients in our database were on nebulized/oral antibiotics as per CF guidelines; however, we acknowledge that there was no methodology to assess ongoing compliance, and this may have been a confounding factor.
•See Page 11, Lines 5-8

Comment 2
It is unclear to me why a regression analysis could not be done, recommend statistical opinion in regard to this. If so are alternatives available to use in the context of a retrospective observational design?
Response 2
We thank the reviewer for this comment, and acknowledge that this was a major design flaw. Accordingly we have performed a regression analysis at each level of severity and have included our results in the revised manuscript.
•See Page 6, Lines 25-30
•See Page 7, Lines 3-16
•See Page 9, Table 3, Lines 24-41
•See Supplemental tables in Appendix

Comment 3
It is also unclear why only those with exacerbations were chosen for analysis, why not all subjects on the database? As it stands this only addresses the application in those who do have exacerbations.
Response 3
We thank the review for requesting this clarification. This study design was aimed at comparing the proportion of HS/non-HS users in all pulmonary exacerbations; hence all exacerbations were initially identified. We acknowledge that another approach would be to compare the frequency of exacerbations in HS/non-HS users using the entire database; this would be a different hypothesis and was not our original intent.

Comment 4
Further analysis to assess confounding of multiple factors should still be possible, I would be guided by statistical review here but certainly pose the question prior to publication.
Response 4
See Response 2

VERSION 2 - REVIEW

REVIEWER	***Scott H. Donaldson, MD***
REVIEW RETURNED	25-Jan-2011

GENERAL COMMENTS	General comments: The authors have heeded some of the reviewer's major concerns, and have performed additional statistical analyses (multivariate logistic regression) of their data. The results of these analyses actually change their conclusions – particularly that in patients with severe disease, the OR of having an exacerbation went from 5.6 to 0.02. Although the addition of the multivariate logistic regression seems to improve the quality of this

	study, I still believe significant clarifications of the design and results is needed, including a formal statistical review. In introduction – the authors don't state what the actual effect of HS was on exacerbations in the Elkins trial, apparently in an attempt to increase the novelty of their own findings. The profound effect on exacerbations was certainly the most important observation in the Elkins trial, even though it was a secondary outcome, and should be reviewed (quantitatively) here. Methods and Results: Is the center an adult or "combined med/peds" center (relevant to age range of included patients)? A clear description of the "groups" and "cases" are still not provided in the text. 340 exacerbations were identified during the retrospective study period – but it's not clearly stated in how many patients these exacerbations occurred. An exacerbation is apparently treated as a "case" – and I would imagine that an individual patient could be in both "groups" if they had an exacerbation both while using, and not using HS at different times. I think the "groups" they compare are those using HS vs. not using HS at the time of an exacerbation, but how this isn't stated clearly either. It would be hard to believe that no patients changed their "group" (i.e. prescribed, or not) during the 3 year that followed the publication on HS in 2006. All of this needs to be significantly clarified. In the stratified assessment of HS effects by lung function, I would again assume that individual patients (with multiple exacerbations) could be counted in different strata, depending upon their lung function at the time of that exacerbation. Do baseline lung function assessments (at initial identification) define a patient's group, or is a value closest to the exacerbation event used? If I add up the number of patients in each lung function group, the total is 424 – is this the total population (counting each patient once) – or is it the sum of lung function classifications (with a single subject counted in more than one group, depending upon lung function measured at the time of the exacerbation)? Does this mean that patients not having an exacerbation are indeed included in the data analysis (as opposed to the "340 cases" they initially say they identified)? Again, clarity is lacking. If a patient they follow at their CF center did not have an exacerbation during the study period, are they completely ignored from the analysis? If so, their conclusions are certainly less generalizable and any conclusions need to be worded appropriately. The effect of HS on completely preventing an exacerbation is impossible to say – only the frequency is reduced in those actively experiencing exacerbations. I would think that this approach of excluding patients without exacerbations will introduce some bias as well. Why weren't all patients included in the analysis to examine the overall effect of HS on exacerbation frequency in various disease severity groups? I still don't know how many exacerbations occurred in each lung

	strata, in users/non-users of HS. Seeing raw and/or adjusted data re: the rate of exacerbations in each group (#/person-year) would be very useful in the assessment of the effect of HS, rather than just odds ratios. Finally, it would seem logical to look at the effect of HS on exacerbations in the overall group (could use FEV1 as a continuous variable if performing a multivariate analysis) before jumping into the subgroups of lung function. I really believe there is some very useful data that is worth publishing here – but we need to make sure that the design is valid (i.e., needs a formal statistical reviewer), and that it is presented more clearly.

REVIEWER	***Peter Wark***
REVIEW RETURNED	04-Feb-2011

RESULTS & CONCLUSIONS	Despite the logistic regression analysis I have concerns regarding the high proportion of subjects in the no treatment arm with Pseudomonas. This is known to affect severity and likely exacerbation frequency. I think this needs to be discussed further in the discussion. it would be helpful to know what independent effect this had on risk of exacerbation. I understand the limitations given the retrospective nature of the data but see real merit in the use of the port cf data in this way.

REVIEWER	***Stephanie MacNeill***
REVIEW RETURNED	23-Feb-2011

THE STUDY	1. Within the paper it is important to clarify who and what are being analysed. From my interpretation I assume the researchers were looking at all patients and among these patients they are assessing the impact of HS treatment on the occurrence of at least one exacerbation. I am commenting on the paper assuming this to be true. I am therefore confused by the researchers' responses to previous reviewer comments which suggest otherwise. If everyone in the study had the outcome of interest how then could a logistic regression be performed? This must be clarified. The following comments are based on the premise that my initial interpretation of the methodology is correct: 2. The patients and Port CF are not described in sufficient detail. In terms of Port CF is important to describe: - who (and who doesn't) appear on Port CF - what data are collected on patients and when (annual review only or all clinical encounters?) - are the data from a single CF care centre or from the national registry? It would appear that it is the former, but this should be explicitly stated since Port CF is a national registry.

	- a description of the care centre would be appropriate – are patients attending this centre representative of the greater CF population? In terms of the patients included in the analysis: - explicitly state how many patients were included and what the inclusion criteria were. Are they only patients identified in 2006 then followed for 3 years? - were there age restrictions in the selection? It is noted that the patients appear to be in their teens and older. - once the number of patients included is identified, it would be appropriate to describe how many of these patients had at least one exacerbation and how many exacerbations were identified in total. From what I understand there are 340 exacerbations in total among 424 patients, but it isn't obvious how many patients were exacerbation-free and how many had at least one. This needs to be made much clearer. 3. When determining whether the patient was receiving HS treatment or not what time point did you look at? Also, when were the other variables under study (age, FEV1, FVC etc) measured? 4. Within the statistical methods section it would be worth stating which variables were included in the multivariate model and how such variables were identified. 5. Within the abstract, it would be helpful to explicitly state that the logistic regression model adjusted for potential confounders. 6. The conclusion drawn in the abstract, summary and discussion need to be toned down slightly. The researchers found that patients on HS were less likely to have exacerbations, but these results alone do not suggest causation. 7. Within the article focus section it should be made clear that points 1 and 2 relate to previously published work by other groups. 8. There are a number of limitations that should be addressed: - completeness of PortCF data – would all exacerbations be recorded? - Missing values – there is no mention of whether complete data were available for all of the variables under study. This should be addressed within the STROBE statement as well.
RESULTS & CONCLUSIONS	9. Within table 1 it is unclear why there isn't a p-value for airway clearance or the two types of exacerbations. Is it the case that the regression models are for a more "global" definition of exacerbation – either hospitalisation OR home IV antibiotics? If so, a row for that should be included as well. 10. Simply scanning through the results of table 1 shows that patients on HS were more likely to have home iv antibiotics and less likely of having hospitalizations. Could this difference be explored? 11. There are 2 table 2s so I assume the second should be labelled table 3. In both cases it would be worth quoting numbers of patients with/without HS treatment and with/without exacerbations. Furthermore, as table 3 is a multivariate model it would be appropriate to include the odds ratios (95% CI and p-value) for the potential confounders included in the model. The tables included in the appendix could be combined into a single table and incorporated within the main body of the paper as table 4.

	12. In the tables currently in the appendix the Wald test statistic is quoted but this is not needed. 13. The researchers highlight that HS was discontinued in only 4 cases with severe lung disease. Were there any patients with mild/moderate lung disease for whom HS was discontinued? 14. As mentioned in comment 6, the researchers should tone down their conclusion that HS treatment is protective against exacerbations.

VERSION 2 – AUTHOR RESPONSE

Response to reviewers for Manuscript # BMJ Open-2010-000019

We wish to sincerely thank the reviewers for the time and effort taken to review this manuscript. We appreciate the opportunity to address issues and concerns raised in a point-by-point manner below and where indicated in the revised paper. All changes in the revised manuscript have been highlighted in yellow text, as well as listed by numerical page and line number below.

Reviewer Wark

We thank the reviewer for requesting this clarification. We agree that the lower prevalence of sputum Pseudomonas positivity in HS-users would likely introduce a confounding effect into the study results, and acknowledge this specifically as a limitation.
• See Page 13, Lines 20-26
The regression analysis would attempt to correct for this inequality in groups, but we fully concur that this is by no means a perfect study design.
As such, this study was not designed to independently examine the effect of Pseudomonas on risk of pulmonary exacerbations.

Reviewer MacNeill

Response 1:
We thank the reviewer for requesting this clarification. The reviewer is correct, the total is 424 patients as depicted in Table 1, out of which 340 had pulmonary exacerbations.
• See Page 7, Lines 45-46

Response 2
We thank the reviewer for requesting this clarification. All patients enrolled in the CF care center are automatically enlisted in the Port CF registry. Data are collected at each visit on all patients. We obtained IRB approval to utilize only our institutional data within the port CF database; the entire registry does represent a nationwide database. Since we collected data from all our patients, we fell that this would be fairly representative of the greater CF population. Also, ALL patients who were enrolled in the database between January 2006 and January 2009 were included; there were no inclusive or exclusive age criteria.
• See Page 6, Lines 47-55

Response 3
We thank the reviewer for requesting this important clarification.
At each exacerbation, it was determined whether the patient was using inhaled HS or not. As far as we could record, there was no reported crossover between HS and non-HS users over time, although we acknowledge that compliance was not well documented. Baseline variables such as FEV1, FVC, etc., were recorded at the first visit to classify disease severity.
• See Page 7, Lines 8-13
• See Page 12, Line 14

Response 4
We thank the reviewer for this comment.
Variables included in the logistic regression were chosen based on the existing literature that describes other known contributing factors to exacerbations, as well as on the differences between the groups at baseline.
• See Page 7, Lines 32-37
• See Page 9, Table 1.

Response 5
The abstract has been restated to include this
• See Page 2, Line 38

Response 6
We thank the reviewer for this suggestion; accordingly we have softened our conclusions in all the requested areas
• See Page 3, Line 3
• See Page 4, Lines 27-31
• See Page 11, Lines 45-50
• See Page 12, Lines 39-42

Response 7
• See Page 4, Line 9

Response 8
It is possible that some exacerbations went unreported; for all recorded cases, complete data was available. This has been stated in the limitations.
• See Page 12, Line 16

Response 9
We thank the reviewer for this comment.
However, we wish to point out that the clinical definition of exacerbation encompasses both home and i.v. antibiotic use, and that separating the two is not clinically relevant. That distinction in the table was at the behest of another reviewer to merely provide more descriptive baseline characteristics of the population under study.
• See Page 7, Lines 5-8

Response 10
We thank the reviewer for this comment.
As mentioned in Response 9, the focus of this study was on the frequency of exacerbations, either with home antibiotics or with hospitalization. It was not the study aim to differentiate between these two clinical scenarios
• See Page 7, Lines 5-8

Response 11
We thank the reviewer for requesting this clarification. Table 3 has been renamed accordingly. We have also included number of cases used inhaled HS and number of pulmonary exacerbations in each table. However, we feel that combining the tables would lead to an unnecessary voluminous tabulation of the data that might prove challenging to the reader who is less statistically inclined. That remains the intent in keeping the individual tables succinct and providing the details in the appendix for the more discerning reader.
• See Page 10, Tables 2 & 3

Response 12
We thank the reviewer for this suggestion; we are advised by our statistician to display the Wald test statistic in the appendix tables for the benefit of the more statistically discerning reader, as mentioned above.

Response 13
We thank the reviewer for suggesting requesting this clarification. There were no other mild or moderate cases that discontinued inhaled HS.

Response 14
We thank the reviewer for this suggestion; accordingly we have repeatedly toned down our conclusions throughout the manuscript.
• See Page 3, Line 3
• See Page 4, Lines 27-31
• See Page 11, Lines 45-50
• See Page 12, Lines 39-42

Reviewer Donaldson

We thank the reviewer for requesting numerous clarifications. As such, the reviewer poses several statistical clarifications that have already been addressed in our responses to the separate aforementioned formal statistical review that the same reviewer has requested.
We direct the reviewer to these responses, but list some clarifications below as well in a point-wise manner
• We have added the quantitative effect of HS in reducing exacerbations in the Elkins trial. See Page 6, Lines 22-25
• The data does represent a combined adult and pediatric population.
See Page 6, Lines 47-48
• 340 cases of exacerbations were identified from a cohort of 424 patients. See Page 7, Lines 45-46
• Severity of lung disease was classified at baseline, i.e., upon initial entry into the CF database.
• We did not record any crossovers between HS and non-HS users in our study period. However, we duly acknowledge that patients may have been non-compliant with HS use at different times over 3 years; and this does introduce bias into our results. See Page 12, Lines 18-19.
• We also agree that identifying the exacerbations initially in a retrospective manner is a study design flaw, and acknowledge this in the manuscript. In this context, we have also scaled down our inferences regarding the role of inhaled HS in reducing the frequency of exacerbations based on our study. See Page 12, Lines 13-16. Also See Response 14 to reviewer MacNeill (above).

VERSION 3 - REVIEW

REVIEWER	***Peter Wark***
REVIEW RETURNED	29-May-2011

GENERAL COMMENTS	Happy for this article to be published. The authors have adequately covered all raised concerns.