Author's response to reviews

Title: Acute Inhalation of Hypertonic Saline does not Improve Mucociliary Clearance in all Children with Cystic Fibrosis

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Author's response to reviews: see over
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Editor-in-Chief
Pulmonary Medicine

Dear Sir:

Enclosed you will find our revised manuscript entitled: Acute Inhalation of Hypertonic Saline does not Improve Mucociliary Clearance in all Children with Cystic Fibrosis. The manuscript and three figures have been significantly rewritten, in response to all of the Reviewers’ comments. All changes to the manuscript are highlighted in yellow.

A point-by-point response to each of the three Reviewer’s comments is presented below.

Thank you for your consideration of this manuscript for publication in Pulmonary Medicine.

Sincerely,

Beth L. Laube, Ph.D.
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Authors’ Responses to Reviewers’ Comments

Reviewer: Editorial Board Member

Reviewer’s Comment #1: There is some confusion in the manuscript about the assessments of MCC. The abstract implies that 3 measurements were made i.e., basal, post placebo (saline) and post hypertonic saline. However, the methods suggest only 2 measurements and the numbers reported for basal and post-placebo are identical. This must be clarified and it is not acceptable to call the post placebo measurement, basal.

Authors’ Response: This has been clarified in the text. We no longer call the post placebo measurement basal. For the children with CF, there were only two assessments of MCC. These were MCC measured on the placebo visit and on the hypertonic saline visit. These two assessments are the only assessments discussed in the revised text and the change is evident in the highlighted text of the Abstract, Methods, Results and Discussion sections.

Reviewer’s Comment #2: I would also like to see the order of the tests discussed. Was the order randomized or did all kids do the placebo measurement first?
Authors’ Response: The order of the placebo and hypertonic visits was randomized by the staff of our Research Pharmacy. This is included in the Abstract and in the Methods section under Study Design.

Reviewer’s Comment #3: Presenting the MCC data as mean and SD may not give the best representation. The data in figure 1 do not appear to be normally distributed and, in any case, I think median and 25-75% is likely to be more useful.

Authors’ Response: The data are now presented as median and 25th and 75th percentiles (i.e. Interquartile Range). Please see the Statistical Analysis section. Figures 2 and 3 have also been revised to show the data as box plots with median and 25th and 75th percentile.

Reviewer’s Comment #4: Suggesting that the CF group had “normal” MCC as there was no statistically significant difference from an adult control group is a bit misleading. Certainly some of the children had very low clearance rates.

Authors’ Response: We agree with the reviewer. We have revised our Conclusions so as to eliminate the suggestion that the CF group had normal MCC because there was no statistically significant difference with the adult control group. We also now point out in the Results and Discussion sections that “10/12 children showed MCC90 values less than the median of 29.6% observed in the adults”. Our conclusions now read: “These data suggest that percent MCC varies significantly between children with CF lung disease and normal pulmonary functions, with some children demonstrating MCC values within the normal range and others showing MCC values that are below normal values”.

Reviewer’s Comment #5: An alternative explanation for the better results in males following HS may be due to the males, on average having poorer MCC. I think an appropriate post-hoc analysis would be to look at improvement in those with lower MCC compared to those with higher values. This would also be more valuable clinically and more intuitive than a sex-based analysis with small numbers.

Authors’ Response: We have refocused our post hoc analysis as recommended by the Reviewer. The post hoc analysis based on gender has been deleted. A new subgroup analysis based on lower versus higher percent MCC values on the placebo visit has been added. The new post hoc subgroup analysis of the change in MCC90 after HS showed a significantly greater improvement in MCC in children with a placebo MCC ≤19.3%, compared to children with a placebo MCC >19.3% (p = 0.045). Please see highlighted language in the revised Statistical Analysis, Results and Discussion sections of the new text.

Reviewer’s Comment #6: Finally the conclusion must change. A group mean value that does not differ from adult controls does not mean that some children may not
have very abnormal MCC that may improve following treatment with HS – as shown in figure 1.

**Authors’ Response:** The conclusions have been changed to reflect the concerns of the Reviewer. The new Conclusion section in the Abstract and the main text now reads: “These data suggest that percent MCC varies significantly between children with CF lung disease and normal pulmonary functions, with some children demonstrating MCC values within the normal range and others showing MCC values that are below normal values. In addition, although MCC did not improve in all children after inhalation of HS, improvement did occur in children with relatively low MCC values after placebo. This finding suggests that acute inhalation of hypertonic saline may benefit a subset of children with low MCC values”.

**Reviewer:** Mark Elkins

**Reviewer’s Comment #1.** "baseline MCC was normal in these children"

This is not necessarily true. A control group of healthy adults differs by two factors - age and disease status. Therefore the fact that the groups had similar baseline MCC is no guarantee that baseline MCC is normal in these CF children. It may be that healthy children should have faster clearance than healthy adults, but we have no data to confirm this.

**Authors’ Response:** Agreed. This statement has been deleted from the revised manuscript. The Conclusions, Results and the first paragraph of the Discussion section have also been rewritten to reflect the reviewer’s concerns.

**Reviewer’s Comment #2.** "not all children with minimal CF lung disease will benefit from acute HS therapy"

HS has other mechanisms of benefit besides accelerating MCC at 60 to 90min. For example, immediate increases in clearance during the inhalation of the HS (which is when most of the effect was seen in the Robinson studies), disruption of existing biofilms, inhibition of new biofilms, etc. Therefore this study has by no means proven that not all children with minimal CF lung disease will benefit from acute HS therapy.

**Authors’ Response:** Agreed. The benefit language for HS in the Conclusions and throughout the text has been revised to apply only to MCC.

**Reviewer’s Comment #3.** "more studies are needed to clarify the clinical significance of the observed gender difference in basal MCC"

There are only 5 subjects in the male subgroup, which is insufficient to determine
whether the assumptions required for conducting the statistical analysis (eg, that the data can be characterised by a mean and SD) are valid. The effect itself seems largely due to artefact from baseline imbalances in the two gender subgroup. This alleged gender difference is precisely the sort of non-intuitive random finding that one expects from post-hoc analyses chosen after observing the data (even if the other contrasts are only observed and not calculated). It deserves one sentence in this paper at most.

**Authors’ Response:** We have refocused our post hoc analysis. The post hoc analysis based on gender has been deleted. A new post hoc analysis based on lower versus higher percent MCC values on the placebo visit has been added. This analysis was recommended by the Editorial Board Reviewer (see Comment #5). The new post hoc subgroup analysis of the change in MCC90 after HS showed a significantly greater improvement in MCC in children with a placebo MCC ≤19.3%, compared to children with a placebo MCC >19.3% (p = 0.045). Please see highlighted language in the revised Statistical Analysis, Results and Discussion sections of the new text.

**Reviewer’s Comment #4.** The sample size calculation is wrong - it dictates 14 not 12. Furthermore, the SDs obtained are larger than those anticipated. Therefore this study appears to be substantially underpowered. It is not possible to further demonstrate this because the authors do not report mean differences with 95% confidence intervals for any of their non-significant results. However, I am fairly certain they would find that these 95%CIs do not exclude their nominated clinically important difference of 5%.

**Authors’ Response:** Our sample size calculation was based on a paired t-test with a known standard deviation of 6, which does result in a sample size of 12 subjects to have 80% power using a two-sided test with Type I error set at 0.05. Given that the observed standard deviation was 12, we agree with the reviewer that the study is likely underpowered. We have addressed this issue in the paragraph on limitations to the study in the Discussion section. In response to the Editorial Board Reviewer’s comments, we are now reporting medians and interquartile ranges, using the non-parametric Wilcoxon signed rank test for our comparisons, and Figures 2 and 3 are now box and whisker plots.

**Reviewer’s Comment #5.** "double blind"

What a joke. The authors have made no attempt to blind the taste of the trial solutions.

**Authors’ Response:** It is true that we made no attempt to blind the taste of the trial solutions. This was not a joke, but was based on the advice of our Research Pharmacy, whose staff felt that the addition of any masking drugs to the solutions to be aerosolized could have unknown effects on MCC. We have added this information to the revised Methods section. In the original limitations paragraph in
the Discussion section we explained why we felt this could still be considered a double-blinded study and our opinion has not changed.

**Reviewer’s Comment #6.** "it is unknown if a single dose study ... can predict the efficacy of long-term treatment with HS in healthy CF patients"

This was not investigated in the study, so what is the relevance of stating this as a conclusion?

**Authors’ Response:** We have deleted this sentence from the Conclusions.

**Reviewer:** John N Tsanakas

**Reviewer’s Comment #1:** Could the authors comment on the possible impact of bacteria colonization between the two studied subgroups, as a factor responsible for the lower MCC in boys?

**Authors’ Response:** We have refocused our post hoc analysis. The post hoc analysis based on gender has been deleted. A new post hoc analysis based on lower versus higher percent MCC values on the placebo visit has been added. This analysis was recommended by the Editorial Board Reviewer (see Comment #5). The new post hoc subgroup analysis of the change in MCC90 after HS showed a significantly greater improvement in MCC in children with a placebo MCC ≤19.3%, compared to children with a placebo MCC >19.3% (p = 0.045). Please see highlighted language in the revised Statistical Analysis, Results and Discussion sections of the new text.

In regards to the Reviewer’s question about the possible impact of bacteria colonization as a factor responsible for lower MCC in one group or another, we are unable to answer this question. This is because studies of MCC in children infected with specific organisms have not been performed. Therefore, although 10/12 children grew at least one organism after culturing their lung sputum, the possible affect of these organisms on MCC in children in this study cannot be evaluated. We have added this information to the limitations paragraph in the Discussion section.

**Reviewer’s Comment #2:** Is the small number of patients included in the study a “weak point” for a reliable statistical analysis?

**Authors’ Response:** The small number of patients is a limitation of the study and we have added this to the limitations paragraph in the Discussion section.