Author's response to reviews

Title: Ventilatory abnormalities in patients with cystic fibrosis undergoing submaximal treadmill exercise test

Authors:

Paloma Lopes Francisco Parazzi (paloma.francisco@hotmail.com)
Fernando Augusto de Lima Marson (fernandolimamarsen@hotmail.com)
Maria Angela Gonçalves de Oliveira Ribeiro (ribeiromago@gmail.com)
Celize Cruz Bresciani de Almeida (ccb.almeida@gmail.com)
Luis Claudio Martins (lclaudio@fcm.unicamp.br)
Ilma Aparecida Pascoal (Ilma@mpc.com.br)
Adyléia Aparecida Dalbo Contrera Toro (dalbotoro@terra.com.br)
Camila Isabel Santos Shivinski (cacaiss@yahoo.com.br)
José Dirceu Ribeiro (jdirceuribeiro@gmail.com)

Version: 3
Date: 12 March 2015

Author's response to reviews: see over
The authors thank the editors and the revisers (Erik Hulzebos, Stephen Bourke e David Ngan) for their suggestions and corrections of our paper. After reviewing, the article was altered and is being submitted for a new analysis. Thank you to all. Please consider our following answers.

Reviewer: Erik Hulzebos

Reviewer's report:

This is an interesting and relevant study objective to investigated ventilator differences between person with CF and healthy controls during exercise.

I have some questions/concerns about this study:

The aim of this study was to evaluate volumetric capnography and cardiorespiratory parameters in patients with CF and healthy controls, at rest and during a 6-MWT.

Are there data available about the validity and reproducibility (error of measurements) of volumetric capnography measurements: [1] in children and adolescent with CF, [2] at rest and during exercise?

ANSWER: To the best of our knowledge there are no studies which contemplate a normality curve (healthy children) and values of volumetric capnography in the
different age groups in pediatrics. Also, there are no studies that aim to verify the validity and reproducibility of the volumetric capnography curves in children and adolescents with CF. There are only four studies published in PubMed who worked with capnography in cystic fibrosis up until 05/20/2015.

Was also a Body plethysmografie measurement (TLC; RV/TLC; IC/TLC........) available? Because there is an (known) association between static hyperinflation and ventilatory "abnormalities" (dynamic hyperinflation) during exercise.

ANSWER: Body plethysmograph measurements were not included in the present study, due to the fact that our reference center does not have the equipment at this time. We believe that future studies should consider this possibility, as correctly observed by the reviser.

Were the healthy control matched for age, body composition, physical activity level,........... Sentence 143: The exercise stress test was perform on a treadmill................. with a reference (sentence 147) to the six minute walking test. What was the reason to do a six minute walk on a treadmill, instead of the official six minute walking field test.

ANSWER: It would be impossible to accomplish the volumetric capnography during the 6-MWT as recommended by the ATS, because the test would have to take place in a 30m hallway. Therefore, the treadmill was the chosen option, for its proximity to the capnograph equipment. The 6-MWT on the treadmill was supervised by the coordinator and the parameters for each patient were analyzed individually, making it possible to give verbal incentives and to measure cardiorespiratory variables such as HR, RR and SaO2.

Sentence 154: Was the professional who did the measurements blinded?
The measurements took place in the cystic fibrosis clinic on scheduled evaluation days. The first author of the study analyzed all the measurements with the help of at least one other co-author, none were blinded.

Sentence 226: It is VE/VCO2 instead of EV/VCO2.

ANSWER: Only the term VE/VCO2 is used in the written text.

Major Revision:

The six minute walking test, according to the ATS guidelines, was not used in this study but a six minute walking on a treadmill with increased velocity. So the title covers not the content of this study.

ANSWER: We agree with the suggestion and we have modified the title to “Ventilatory abnormalities in patients with cystic fibrosis undergoing submaximal treadmill exercise test”.

No objective or subjective criteria were reported, in both groups, about the rate of perceived exertion of this exercise test. In other words was the exercise intensity in both groups the same?

ANSWER: The intensity of the exercise was according to the tolerance of each individual. The treadmill speed was raised every minute with the patient’s permission. Also, the Borg scale was used, pre and post-test.

No data reported about the breathing pattern (tidal volume; breathing frequency; breathing depth (TV/VC); hyperinflation (IC/TLC) during exercise. Supplementaries have no additional value.
ANSWER: All children completed the test without presenting cardiorespiratory symptoms that would demand its interruption. The results of the variable analysis are described in the results of the paper.

Minor revision:

Please Patients first language: Patients with CF instead of CF patients (e.g. sentence 122, 130,......).

ANSWER: The correction was accepted and the text modified.

Please use the correct abbreviations. For example sentence 86 dead space/tidal volume ratio is not (DS/TV) but (Vd/Vt), EV/VCO2 instead of VE/VCO2,........

ANSWER: The correction was accepted and the text modified.

Sentence 86-87: Performance on the exercise? Please explain this? What are the objective and subjective criteria for exercise performance?

ANSWER: Exercise performance is reduced in patients with CF, due to the chronic pulmonary disease, which changes parameters of pulmonary function. Therefore, the objective criteria for a good exercise performance were considered through analysis of cardiorespiratory variables, such as: SaO2, HR and RR, and the subjective criteria was the Borg scale. Both are described in the study.

Sentence 89: What means the exercise in CF? Exercise training or exercise testing, please be specific.
ANSWER: The phrase 89 refers to the benefits of physical exercise in patients with CF, and was rewritten in the text for a better understanding.

Sentence 109-110. Not all the readers are familiar with VCAP, please explain the (clinically) relevance of a higher value of slope3/TV?

ANSWER: Suggestion accepted and the text was modified to provide better understanding. An increased slope3/VT reflects the inhomogeneity of ventilation in distal airways, suggesting the presence of chronic structural dysfunctions as well as reversible acute dysfunctions that may be observed, for example, in a broncoprovocation test. This index may be a tool in the evaluation and study of the small airway dysfunctions in children and adolescents with pulmonary disease.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published.

ANSWER: The article was sent for a review and had the language corrected by a translator.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Reviewer: Stephen Bourke

Reviewer's report:
This paper provides detailed data on exercise physiology in patients with cystic fibrosis compared to healthy control subjects, and suggests that volumetric capnography in particular might prove to be more sensitive in predicting subclinical ventilatory changes than standard FEV1 and clinical parameters. It is not surprising that patients with lung disease have differences in exercise ventilation when compared to healthy control subjects. The data presented provides some useful baseline data, but for it to be of clinical relevance it would be necessary to see in a longitudinal follow study, whether such changes predicted clinical progression.

Major revisions:

1. The title of the manuscript text refers to the 'six minute walk test' (which is an error, referring to a different exercise test) whereas the submission refers to 'submaximal exercise test 6 minutes' (which is probably what is intended).

   ANSWER: We agree with the reviser's suggestion and have modified the title to “Ventilatory abnormalities in patients with cystic fibrosis undergoing submaximal treadmill exercise test”.

2. Some discussion of whether the results indicate impaired lung function rather than reduced fitness and training effect, would be useful.

   ANSWER: The authors appreciate the questioning. The children were questioned as to their exercise regimen, and both groups only had regular physical activity during physical education classes, and even though that showed uniformity between the groups, we considered it reflected a poor interest and practice of physical activity.

Discretionary revisions:
1. Consider additional analysis of the data within the CF patients across the age spectrum, to see if the results can be related to the stage of the disease in terms of progression of cystic fibrosis lung disease.

ANSWER: The present study did not aim to evaluate the differences between age groups in patients with CF. As described by the reviser, we do have an age variation, but if we split the group and analyze it in smaller age groups we lose the power of the sample’s size and the focus of the study would be altered. For every new analysis we would have much more information to describe in the results and to discuss. The analysis was charted by age and gender in the CF group. All data are charted and not presented in the final paper. They are in the database and may be used in the future for publishing, if we are able to increase the number of patients in the sample.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer: David Ngan

Reviewer's report:

(Report submitted as an attachment.)
I was invited to provide a statistical review for this manuscript submitted to BMC Pulmonary Medicine by Paloma Lopes Francisco Parazzi and colleagues. Parazzi et al. explored the use of volumetric capnography for the assessment of lung function in cystic fibrosis (CF) patients, particularly pertaining to ventilatory efficiency during the
6-minute walk test (6MWT). Since volumetric capnography measures the concentration of carbon dioxide exhaled as a function of volume, this may be useful for the evaluation of pulmonary deterioration in CF patients with respect to tissue damage and dead space.

Major Compulsory Revisions

1. In Figures 1-4 (and Supplementary S4), the authors defined 5 phases of the 6MWT for their analyses: (1) baseline / pre-test, (2) the first 2 minutes of the 6MWT, (3) the third and fourth minutes of the 6MWT, (4) the fifth and sixth minutes of the 6MWT, and (5) post-exercise. The authors stated that they analyzed these data using the Kruskal-Wallis test. This is inappropriate.

ANSWER: The argument stated is correct, and we apologize for the misshapen in the presentation of this study. The test used for statistical analysis was the Wilcoxon test. The data did not present normal distribution and the test cited was used at one point. For the same sample, other analyses were made by the same group of co-authors. In one of the analysis four groups were analyzed: male patients of CF and female patients of CF, male controls and female controls, and in this situation the Kruskal-Wallis test was used. This data is not presented in this study and is not part of the paper forwarded for publishing due to the already high amount of data and information in the study. As to inform the correct test used, in the method section of this study, the correct test is cited. Because the same patients underwent measurements before the exercise test, at 3 intervals during the exercise test, and then after the exercise test, this actually represents a repeated measures design and must be treated as such. Moreover, in the authors’ Discussion (lines #262, 286, 296, 325), they make reference to comparisons “before and after”
exercise, so it seems that their intent was to have a repeated measures analysis. The figure legends of Figures 1, 3, and 4A suggest that the authors would like to analyze between groups (CF patients vs. healthy controls) and also within the time course (Moment 1 vs. Moment 2 vs. Moment 3 vs. Moment 4 vs. Moment 5).

**ANSWER:** The data was analyzed taking into consideration the comparison of the CF group with the Control group, as well as the comparison made between the group itself, in the 5 different moments, as described by the reviser. To us, an important factor, besides the capnography parameters measured during the test, is the variation of the data between the patients and the control group. The moments of highest importance to answer that question are the baseline moment, which demonstrate the dysfunctions simply from the disease, without exercise; and the moment 5 (post-test) which presents the values after the exercise test. The execution of the exercise test is important, and was analyzed individually for each group and moment, and this data is presented in the study as a follow-up of the capnography. But the primary question of the study was about the difference between patients with CF and the control group during exercise, and the step by step analysis of the moments.

- For the comparison between groups, the authors should perform a mixed ANOVA, which is similar to a two-way repeated measures ANOVA but allows for completely separate groups.

For the mixed ANOVA in SPSS (Analyze>>General Linear Model>>Repeated Measures…), the authors would use their current y-axis measures (such as “Respiratory rate”, “Heart rate”, and “SpO2”) as the dependent variable, group (CFG or CG) as the between-subjects factor, and time (Moment 1, Moment 2, Moment 3, Moment 4, and Moment 5) as the with in subjects factor.
• For the comparison within the time course, the authors should perform a one-way repeated measures ANOVA, which allows for the analysis of measurements over several time points.

For the repeated measures ANOVA in SPSS (Analyze>>General Linear Model>>Repeated Measures…), the authors would use their current y-axis measures (such as “Respiratory rate”, “Heart rate”, and “SpO2”) as the dependent variable and time (Moment 1, Moment 2, Moment 3, Moment 4, and Moment 5) as the within-subjects factor.

ANSWER: The analysis described by the reviser is of extreme importance and show the variation between the groups and the moments analyzed in the present study. The analysis model used in our study compares each moment individually, in the control group and the patient group. The analysis that was executed by our group answers to the question/aim of the study completely, although it demands more work during execution.

2. In the legend for Figure 3 and on line #234, the description of Figure 3D is an analysis of DS/TV (dead space to tidal volume ratio). This does not match the actual panel D (“End-Tidal carbon dioxide”); in fact, panel D is exactly the same as panel C and the authors may have inadvertently copied and pasted it there instead of the correct panel (which is missing).

ANSWER: The authors apologize for the misshapen and the new image has been attached to the panel with the correct information.
3. In the Results section on line #220, the authors state that the “RR was higher in CFG than in CG at all times analyzed” and refer to Figure 1. In the figure legend for Figure 1A, the authors state “there was no difference between groups for the periods analyzed.” This is a discrepancy that needs to be corrected.

**ANSWER:** The authors agree with the reviser and the text was changed. In fact, the data was not higher in the CF group, although not statistically significant, as observed in Image.

4. In the Results section on line #222, the authors state that the “SpO2 was lower in CF patients” and refer to Figure 1C. In the figure legend for Figure 1C, the authors state “there was no difference between groups for time 4 and 5.” The authors should add this to the text.

**ANSWER:** The authors agree with the reviser and are thankful for the correction. The changes were made and the complete information has been included in the text.

5. On line #239, the authors state that EV/VCO2 was inversely associated with FEV1(%) “in all times, for CG and CFG” and refer to Figure 4B. Figure 4B and 4C
only show Moments 1 and 5, respectively, according to the figure legend. In addition, the figure legend states that CG was not significant for Moment 1 (p=0.1756, line #601) and CG was not significant for Moment 5 (p=0.1446, line #603). The authors need to correct this discrepancy.

**ANSWER:** The authors agree with the reviser and are thankful for the correction. The changes were made and the complete information has been included in the text.

Minor Essential Revisions

6. On line #509 (the footnotes of Table 1), it is indicated that sex is presented as “absolute value (percentage)”. However, in the corresponding line of the table (“Gender”), the percentages are missing and should be included.

**ANSWER:** The changes were made to the image.

7. In Table 1, the “Borg initial” percentages are reported incorrectly for the “Patients with cystic fibrosis” (0.75% 75%, 0.19% 19%, 0.05% 5%, 0.01% 1%) and need to be corrected.

**ANSWER:** The changes were made to the image.

8. In Table 1, the “Borg end” percentages are reported incorrectly for both “Patients with cystic fibrosis” and “Control” (0.52% 52%, 0.09% 9%, 0.22% 22%, 0.03% 3%, 0.01% 1%, 0.88% 88%, 0.063% 6.3%) and need to be corrected.

**ANSWER:** The changes were made to the image.

9. There is a typographical error in the figure legend of Figure 2 (line #547) for a reported slope: “0,1,248 x”.

**ANSWER:** The changes were made to the image.
10. In Figures 2, 4B, and 4C, the authors should report the R2 value for the linear regression analyses, as the fit of the line to the data would be informative.

**ANSWER:** The authors appreciate the statement and the R2 was included. We redid the whole statistics work following the proposed model, and one of the graphs was wrongly presented, and we have included the correct graph in the final version (Image 4C).

11. Figure 2D is missing confidence intervals displayed on the graph.

**ANSWER:** The authors appreciate the suggestions and the changes were made as follows:
12. There is a typographical error in the figure legend of Figure 4 (line #595) and line #286. The authors likely meant “CG” rather than “GC”.

**ANSWER:** The changes were made to the image.

13. There is a typographical error on line #309. The authors likely meant “CFG” rather than “GFC”.

**ANSWER:** The changes were made to the image.

14. There is a typographical error in the figure legend of Figure 4 (line #603) for a reported slope: “-0.1869” needs to be followed by an “x”.

**ANSWER:** The changes were made to the image.

15. For consistency, the authors should consider swapping the colours and shapes of the data points between the CFG and CG groups in Figure 2, since in all the other figures, blue circles represent CG and red squares represent CFG. In Figure 4B and 4C and Supplementary S4, a legend to identify the colours and shapes needs to be added, and similarly the authors should consider using blue circles for CG and red squares for CFG for consistency.
16. Figures 3 and 4 need letter labels for each panel (A, B, C, D).

ANSWER: The changes were made to the image.

17. There is a typographical error in the figure legend of Supplementary S4 (line #692) for a reported slope: “12,573 x”. The comma should be a decimal point.

ANSWER: The changes were made to the text.

18. There is a typographical error in the figure legend of Supplementary S4 (line #694) for a reported slope: “11,854 x”. The comma should be a decimal point.

ANSWER: The changes were made to the text.