Author's response to reviews

Title: Pulmonary Function in Patients with Huntington's Disease

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Author's response to reviews:

MS: 6488396111186584
RE: Pulmonary Function in Patients with Huntington's Disease.

Dear Ms Ma. Celine Zapanta

Thank you very much for giving us the opportunity to respond to the reviewers' comments and suggestions and revise the manuscript. We have responded to all of the points raised by the reviewers. We have revised the manuscript accordingly, and provided our responses (shown in Italics) to their comments in this letter. In the manuscript, the changes are indicated in blue.

We hope that the revised manuscript will be reviewed by the original reviewers, and you and the reviewers will find that the responses are adequate and the revised manuscript is acceptable for publication in the journal.

Your assistance is greatly appreciated.

Yours sincerely,
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Response to Reviewer # 1

RE: MS 6488396111186584 Pulmonary Function in Patients with Huntington's Disease

Dear Reviewer # 1

Thank you very much for reviewing our manuscript and for the valuable comments and suggestions.
We have responded to all of the points raised by you and other reviewer, revised the manuscript accordingly, and provided our responses (shown in Italics) to your comments in this letter.

In the manuscript, the changes are indicated in blue.

We hope that you will find that the manuscript has been improved. Your further review of the revised manuscript is much appreciated.

Comments and Responses:

1. In the Introduction (p. 4 line 7 and 8) the authors state that no information on respiratory function in HD is available. There is a study on HD and pulmonary function (A PILOT STUDY ON RESPIRATORY FUNCTION IN PEOPLE WITH HUNTINGTON’S DISEASE Jones et.al. J Neurol Neurosurg Psychiatry 2010 81: A42-A43). This study is only presented as an abstract. However it makes the statement disputable. The authors might consider to correct the writing.

We thank the reviewer for providing the information. We overlooked the study, so we have included it in the revised manuscript. We have revised the statement (please see page 4, lines 9-12).

2. A question mark arises on the reference values used (Stanojevic 2009 prediction equations, p. 7). The statement lacks a reference and it is not clear if these equations contain normal values for both FVC and SVC. Further it is not clear why the researcher selected Stanojevic equations and why they are for the population being tested. Since the study has a case-control design reference values have importance mainly for valuing the pulmonary function in the control group. The reference values also have implications when assessing a restrictive pattern and determining chronic airway obstruction when using LLN (Lower limit of normal) or when determining the degree of obstruction. The authors could consider clarifying this.

In the revised version of the manuscript, we have included the reference for the statement regarding the prediction equations used in our study. (Please see page 4, lines 9-12). According to Stanojevic’s study [Stanojevic et al. Am J Respir Crit Care Med Vol 177. pp 253–260, 2008], the equations contain normal values for FVC and FEV1. We used Stanojevic equations because the study of Thompson et al. (2011) showed that these all-age spirometry reference equations fit a contemporary Caucasian Australasian population, which is the population included in our study. The study of Thompson et al. (2011) also suggests that these equations can be applied widely in the clinical setting [Respirology (2011) 16, 912–917]. We agree with the reviewer that reference values are of importance to characterize pulmonary function in the control group of healthy volunteers and to determine the level of pulmonary dysfunction in the HD group. In the revised version of the manuscript we have clarified that the prediction equations include normal values for FVC and FEV1. We also clarified the reason for using these reference values (Please see page 8, lines 1-2).

3. The authors could make it more explicit that the lung function parameters
affected by HD probably only depends on muscle weakness and not to a pulmonary disease since basically only parameters related to forced respiration are affected. Thus a possible pattern of restriction or chronic obstruction really mirrors a neuromuscular disease and probably to a lesser degree lung diseases.

We agree with the reviewer that it is highly possible that the reduced pulmonary function shown in the group of HD patients is caused by respiratory muscle weakness not by a pulmonary disease. In the discussion, we did not make this explicit because in the study we measured maximum inspiratory and expiratory pressures, which are indirect methods for testing respiratory muscle strength. However in the revised manuscript we have clarified the association between the pulmonary dysfunction and respiratory muscle weakness observed in our study (Please see page 13, lines 13-16).

4. The aim is clearly defined but in such a general manner (“to compare”) - that the research question (hypothesis) is concealed (p. 4).

In the revised version of the manuscript we have clarified the hypothesis of our study (Please see page 4, lines 23-25 and page 5, line 1).

5. The authors avoid assessing prevalence of COPD in the groups. This is probably deliberate, but not expressed. Based on the lung function data presented in table 3 it is possible that more subjects in the HD group have a spirometric pattern of COPD with a FEV1/max(FVC SVC) < 0.70. Since the spread of SVC is larger in this group and FEV1 lower than in the control group. It is also possible that the HD group show more of restrictive spirometry since both SVC and FVC of normal are obviously low. The variables of lung function in the study make it possible to determine prevalence of spirometric pattern of COPD and its stages and also of restriction. The authors could either comment on omitting these analyses or include them with commentaries. The authors refer to ATS/ERS standards for spirometry but do not include a reference (the reference to ATS/ERS - 14 - is concerned with respiratory muscle testing). The authors do not declare if bronchodilator (reversibility test) was used. The manuscript could include this information.

We agree with the reviewer that it is relevant to consider the prevalence of COPD in both the control and HD groups. Although there is a history of smoking for some participants from both groups, none of them had a confirmed diagnosis of COPD or presented any known COPD symptoms such as cough, sputum production or dyspnea at the time of the assessment. In the revised version of the manuscript we have specifically included a sentence stating that participants with COPD were not considered/eligible for the study (Please see page 5, lines 23-25 and page 6, lines 1-2). We have also specified those participants with a spirometric pattern of COPD as well as those with a restrictive pattern (Please see the results section in page 10, lines 9-13)

We have included a relevant reference regarding the ATS/ERS standards for spirometry (Please see page 7, line 18).

Given that none of the participants of the study had a confirmed diagnosis of
COPD or presented with any known COPD symptoms at the time of the assessment, a reversibility test with bronchodilator was not necessary. This has been clarified in the revised manuscript (please see page 8, lines 3-6).

6. The statement in the Discussion (conclusion) on age (p. 9) is misleading. The control group is age-matched and reference values are age-dependent. Lung function decline is certainly age-dependent. The statement depends on an “in-group” (sub-group) analysis (data not presented in the article). Is it that the authors refer to HD subjects not showing increased ageing in lung function parameters? This writing should be revised.

We agree with the reviewer that the statement regarding age dependency and lung function decline is misleading. Our initial interest was to exclude any possible confounding factor in the analysis of the results. In the revised version of the manuscript we have removed the statement from the discussion and results sections.

7. The authors hint at clinical implications of the present study (p. 12 line 8-9 and in the Conclusion section). Early detection of respiratory deficiencies is said to be important to prevent severe respiratory complications. It is of great interest if the authors could be more precise in what ways this could be of value for physicians and for patients with Huntington’s disease.

The detection of pulmonary function deficiencies early in the disease course may enable early therapeutic intervention such as respiratory muscle training, manually assisted coughing or mechanical cough assistance. A desirable benefit from such interventions in HD patients is to improve cough function, which can impact by decreasing the risk of aspiration pneumonia. We estimate that the benefits obtained from these interventions would have a higher impact in HD patients at early stages of the disease when muscles have not yet deteriorated beyond repair than that at late stages when muscle and neural damage is extensive. In the revised version of the manuscript we have specified the importance of early detection of respiratory function deficiencies for HD patients (please see page 14, lines 6-13).

8. There are previous studies on HD with some similarities. There is a study on muscle weakness in HD showing lower limb strength (published in full) and a study on lung function decline in HD, only in abstract form (Jones 2010). Neither of these are referred to.

We appreciate the information about the references. The study by Jones et al. (2010) has been included and referenced in the introduction of the manuscript (please see page 4, lines 9-12). If we understand correctly, the study by Busse et al. (J Neurol 255:1534–1540, 2008) did not measure respiratory muscle strength, thus we did not feel that their results are relevant to our study.

Response to Reviewer # 2

RE: MS 6488396111186584 Pulmonary Function in Patients with Huntington’s
Disease

Dear Reviewer # 2

Thank you very much for reviewing our manuscript and for the valuable comments and suggestions.

We have responded to all of the points raised by you and by the other reviewer. We have revised the manuscript accordingly, and provided our responses (shown in Italics) to your comments in this letter.

In the manuscript, the changes are indicated in blue.

We hope that you will find that the manuscript has been improved.

Your further review of the revised manuscript is much appreciated.

Comments and Responses:

1. With an expected difference of 30 cmH2O difference in MEP between groups (SD=30), alpha=0.05 and 80% power, I argue that the sample size should be at least 13 participants per group, not 12 (as described in the Participants section). Please elaborate on why you base the sample size calculation on the largest expected difference? I also suggest that the sentence “To achieve statistical significance of 5% and a statistical power of 80%, an adequate ... ...” is revised into the more accurate “With alpha 0.05 and 80% power, an adequate ... ...”. And in connection to this matter, do you have sufficient power to make comparisons of mean pulmonary function values between subgroups such as e.g. smoking subgroups of HD patients (n=9 per group) as described in the Result and Discussion sections?

We agree with the reviewer that the sample size should be at least 13 participants instead of 12. We have corrected the result of the sample size calculation in the revised manuscript (please see page 5, line 15). We based our sample size calculation on the largest expected difference because in HD, the main cause of death is an aspiration pneumonia event, which is often associated with incapacity to clear airway secretions. Given that expiratory muscles have an important role in clearing airway secretions we considered that MEP was the most indicative parameter of pulmonary deficiencies in HD. In the revised version of the manuscript we have clarified the reason why we chose MEP to calculate the sample size (please see page 5, lines 10-13).

We have also revised the sentence detailing the statistical significance and power as suggested (please see page 5, lines 14-16)

We agree with the reviewer that there is not sufficient statistical power to make comparisons of pulmonary function between ex-smokers and non-smokers in the HD group. Our interest was to exclude any possible confounding factors that may be responsible for pulmonary function differences between individuals with HD and healthy volunteers. In the discussion section of the revised version of the manuscript we have mentioned the lack of statistical power in this comparison (please see page 11, lines 18-22).
2. The description of the sampling of the control group is insufficient. Since there are several factors, which can affect lung function, which are not accounted for in the exclusion/inclusion criteria, please explain from where the controls are sampled. Are they sampled from hospitals, specific work places or other areas? Factors such as e.g. occupational exposure to gas/dust/fumes (related to lower lung function) or high socioeconomic status (related to higher lung function), which possibly could be overrepresented among controls may be a confounding factor not described in the paper. Also, please elaborate on why the gender (sex) matching was not exact between HD patients and controls.

Thank you for raising this important issue. The healthy volunteer group mainly consisted of partners of the HD patients, as such they had similar life-style background to the patients. None of the healthy volunteers had occupational or ambient exposure to pollutants (except for those who were exposed to smoke) that could affect pulmonary function. In the revised version of the manuscript we have clarified the exclusion criteria and description of the control group (please see page 5 lines 23-25, page 6, lines 1-2 and page 6 lines 4-7). Despite best efforts, we were unable to recruit healthy volunteers to match exactly the gender between groups.

3. Please consider displaying 95% Confidence Intervals for the mean values in table 2 and 3.

We have eliminated the ranges and included 95% confidence intervals for all variables in Tables 2 and 3 (please see Tables 2 and 3 in pages 20 and 21).

4. Why did you choose the Stanojevic 2009 prediction equations as reference values for spirometric values, and not the GLI-2012 equations, which currently are recommended by most recognized respiratory societies? Both the Stanojevic and the GLI reference values are evaluated on a population sample from Australia/New Zealand and found to be applicable. And, why is % of predicted not presented for the FEV1/FVC ratio? Additionally, does the Stanojevic reference value for SVC predict Slow Vital Capacity or the highest value of Forced and Slow Vital Capacity? Also, please provide a reference to the Stanojevic reference values (describing all spirometric indices) where the reader can find this information.

Stanojevic 2009 and GLI-2012 prediction equations are both validated in a Caucasian Australasian population and are applicable and recommended by the ATS and ERS. The software (BreezeSuite 7.1.0.54 SP3; copy date: 10/2011) incorporated to the spirometer (Medgraphics, model CPFS/D, St. Paul MN, USA) that we used in our study, did not include GLI-2012 prediction equations.

In the revised version of the manuscript we have specified that Stanojevic 2009 prediction equations include normal values for FVC and FEV1 and are validated in a Caucasian Australasian population, which is the population utilized in our study (Please see page 8,lines 1-2).

We estimated that the FEV1/FVC ratio presented as percentage of predicted is
not an informative index to describe pulmonary function. On the contrary, the ratio of the two absolute volumes (FEV1/FVC) is widely used as diagnostic spirometry criteria for asthma, COPD and other respiratory conditions [Allen et al. Predicting inadequate spirometry technique and the use of FEV1/FEV3 as an alternative to FEV1/FVC for patients with mild cognitive impairment. The Clinical Respiratory Journal 2008; 2: 208–213.]

In the revised version of the manuscript, we have provided a reference as requested by the reviewer (please see page 7, line 25).

5. The controls have mean values well below 100% for the spirometric measurements of SVC, FVC, FEV1 and PEF although mean % of predicted is expected to be close to 100% for healthy subjects. Is the relatively low mean % of predicted values for the control group due to the fact that six controls have a smoking history? In essence, do the former smokers “lower” the mean (even though the subgroup analysis did not yield significant differences and mean time elapsed since smoking cessation>20 years)? Or may some other factor influence their spirometric performance?

Effectively the 6 ex-smoker participants in the control group have lower the mean values of SVC, FVC, FEV1 and PEF in comparison to those in the 12 non-smoker group. In the revised version of the manuscript we have addressed this issue (please see page 11, lines 22-25 and page 12, lines 1-3).

6. Table 1 gives the impression that all participants had a smoking history, i.e. values for packyears and time elapsed from smoking cessation, when in fact only nine HD patients and six controls had values for these variables. This should be explained in the table. Also, do you have sufficient power to test for differences in these variables between HD patients and controls, and are the two variables normally distributed?

We have specified in the table, the number of people who had smoking history and the time elapsed from smoke cessation (please see Table 1 in page 19). The comment regarding the statistical power is elaborated in the response for comment #1. Both variables are normally distributed. The p-values are presented below for your perusal.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time elapsed from smoke cessation</td>
<td>0.887</td>
</tr>
<tr>
<td>Pack-year</td>
<td>0.572</td>
</tr>
</tbody>
</table>

7. In the Participants section, please consider revising the sentence “Eighteen patients with manifest HD ...... scheme database [10], and the Huntington’s Western Australia Association”. One possible suggestion is: “Eighteen patients with manifest HD ...... scheme database [10], with assistance from the Huntington’s Western Australia Association”.

We have revised the sentence and made the change as suggested by the reviewer (please see page 5, lines 18-19).
8. In the Methods section, page 5, second paragraph: one inclusion criterion is stated as a clinically verified disease expression of UHDRS-TMS<=5. This should be changed into UHDRS-TMS>=5.

We have made the change as suggested by the reviewer (please see page 5, line 21).

9. Please explain how height was estimated. It is important that height is measured at the time of investigation and not estimated merely by asking the participant, preferably by using an accurate and calibrated stadiometer, since self-reported height and weight can be seriously biased. An accurate height estimate is important in order to enable calculation of an accurate reference value. The same goes for weight when using the Stanojevic reference values.

Height and weight were not estimated but actually were measured for all participants at the time of assessment using an accurate and calibrated scale and stadiometer. The methods of measuring height and weight are clarified in the revised manuscript (please see page 8, lines 12-14).

10. The packyears term generally describes the cumulative number of cigarette pack equivalents consumed by a subject up to time of examination, and is usually spelled as either “packyears” or “pack-years”. You use the term “pack/year” throughout the paper, which gives the impression of number of packs per year, which is not the purpose of this term. Also, in the footnote to Table 1, you use the term “Pack year”. Please change this throughout the paper.

In the revised version of the manuscript, we have changed the term pack/year to pack-year (please see page 11, lines 13 and 15; and the footnote to Table 1 in page 19).

11. Was the Shapiro-Wilk test performed for all variables and in all subgroups where statistical tests are performed? Please clarify.

The Shapiro-Wilk test was performed for all variables in all subgroups. This is now specified in the revised version of the manuscript (please see page 9, lines 19-20).

12. Since the significance level is stated in the Statistical Analysis section, you are not required to state both p-value AND “statistical significant” throughout the text, when describing statistically significant differences between groups. Preferably, “(p<0.05)” can be removed in those sentences, e.g. in the Results section, page 9, second paragraph.

We have made the changes as suggested by the reviewer throughout the text in the revised manuscript.

13. Please provide equal number of decimals (preferably 2) for mean height and range for height in Table 1. The same goes for number of packyears in Table 1.

Thank you for pointing out this inconsistency. We now have included the same
number of decimals for mean height, range of height and pack-years in Table 1 (please see Table 1 in page 19).

14. Please add that the correlations are calculated for UHDRS-TMS and spirometric indices expressed as % of predicted (not for absolute values) to the figure legend of Figure 1.

This specification has been included in the legend for Figure 1 (please see the legend for Figure 1 in page 23)

15. Please consider revising the first sentence in the first paragraph of the Results section, page 8. It should be clear that the groups you refer to are the HD patients and controls. This can be inverted in the second sentence.

We have revised the two sentences (please see page 9, line 13 and line 16).

16. Regarding the second last sentence in the Results section, page 9; is this sentence referring to Figure 1? More specifically, is this paragraph referring to absolute values or % of predicted? Please clarify.

The sentence mentioned by the reviewer makes reference to Figure 1. This specification has been made in the revised version of the manuscript (please see page 10, line 20). The paragraph is referring to spirometric indices presented as % of predicted values. We have clarified this in the manuscript (please see page 10, line 18).

17. Regarding the last sentence in the Results section, page 9; were the correlations between age and the pulmonary function variables calculated for both absolute and % of predicted values or only for % of predicted? One would certainly expect a significant correlation between age and absolute values. And was this the case among both HD patients and controls? If no correlations with age were found neither for HD patients nor controls, you cannot suggest that this finding is specific for HD patients (as implied in the Discussion section on page 9). Please elaborate on this matter.

The correlations between age and pulmonary function variables were calculated using both absolute and % of predicted values and for HD and control groups. We agree with the reviewer that the statement regarding age dependency and lung function decline is misleading. Our initial interest was to exclude any possible confounding factor in the analysis of the results. In the revised version of the manuscript we have removed the statement from the discussion and the results sections.

18. In the Discussion section, page 10, please consider revising the sentence “In the present study, however smoking history was under 20 pack/years for HD and control groups” and add the word mean into the sentence, e.g. into “In the present study however, both HD patients and controls had mean packyears of smoking less than 20”.

We have made the change as suggested (please see page 11, lines 14-15).
19. Consider revising the expression “No information is available” (regarding respiratory function in HD patients), which is used in the Abstract and in the Introduction section. Is there truly no information at all available, or should it maybe me more appropriate to express the matter as e.g. “To our knowledge, no publications are available”?

We agree with the reviewer that it is better to put “to the best of our knowledge” and in fact that the statement was not correct. In fact other reviewer provided a reference [Jones U. et al. Journal of Neurology, Neurosurgery & Psychiatry 2010, 81:A42-A43.]. In the revised manuscript we have modified the sentence (Please see page 2,line 6 and page 4, lines 9-12). Moreover we have included the reference by Jones et al. (2010) in the introduction (Please see page 4,line12).

20. Can the terms pulmonary function and respiratory function be used interchangeably, as done in the Introduction section?

We consider that the terms pulmonary function and respiratory function should not be used interchangeably. We have changed the term “respiratory function” to “pulmonary function” (please see page 4,lines 9-12).

21. In the Result section, page 8, please consider revising the sentence “However, MIP values across testing sessions were more variable in the HD (CV 10.8%) than in the control group.....” into “However, the variability for MIP values across testing sessions was larger in the HD (CV 10.8%) than in the control group.....”.

We have revised the sentence as suggested (please see page 9, lines 24-25).