Author's response to reviews

**Title:** 25-hydroxyvitamin D deficiency, exacerbation frequency and human rhinovirus exacerbations in chronic obstructive pulmonary disease

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**Author's response to reviews:** see over
Dear Dr. T Shipley,

Thank you very much for providing me with the opportunity to submit a revised manuscript and to both reviewers for the very helpful comments. I have addressed each of the reviewers’ comments below and revised the manuscript as requested. My changes and responses are all in red.

Reviewer: Marco Contoli
Reviewer’s report:
In the manuscript titled “25-hydroxyvitamin D deficiency, exacerbation frequency and human rhinovirus exacerbations in chronic obstructive pulmonary disease” the authors aim to investigate whether deficient vitamin D is linked to increased exacerbation frequency in COPD. The topic of the study is relevant and it has been performed by a group with solid experience in the field. The authors found that low vitamin D levels are not associated with exacerbation frequency. I have only few minor comments to address to the authors.
1) The results are in line with a very recent large study (Kunisaki et al. AJRCCM 2012) showing that in patients with severe COPD, baseline vitamin D levels are not predictive of subsequent exacerbation. The authors should include and mention this study in the discussion. This study has now been included and referenced in the discussion.
2) At page 11, line 251. The authors conclude: “The next step in establishing the importance of 25-hydroxyvitamin D deficiency in COPD is to study supplementation in adequately powered clinical trials using relevant clinical outcomes.” This has been very recently performed in a single-centre, randomised, double-blind, placebo-controlled study (Lehouck et al. Ann Internal Medicine 2012). The study shows that high-dose of vitamin D supplementation in COPD patients does not reduce the incidence of exacerbations. However, the same study also documents that in participants with severe vitamin D deficiency at baseline (post hoc analysis) supplementation may reduce exacerbations. The authors should quote and address this study in the discussion. This study has now been included and referenced in the discussion.
3) The authors used the following definition to capture an exacerbation: “increase of two symptoms (at least one major) for two consecutive days, or if in the...
opinion of the attending clinician.” Did the author evaluate severe exacerbation frequency (i.e. requiring antibiotics or systemic corticosteroids) in relationship with vitamin D levels? All exacerbations in this study were treated with antibiotics and steroids. None of the exacerbations required hospitalisation. This has been clarified in the text.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer: Alice Wood
Reviewer's report:
I have structured my report according to the questions asked of reviewers for BMC Pulm Med, and then listed revisions requested in the 3 categories stated.
1. Is the question posed by the authors well defined?
There are a number of questions clearly stated by the authors in the background section, although it is not made clear whether all were equally important or one of them was the primary outcome measure; I have assumed that the first stated question was the outcome of most interest for the purpose of this review. These questions are as follows:
(a) Are COPD patients deficient in 25-hydroxyvitamin D more likely to be frequent exacerbators, relative to those with insufficient or normal levels?
(b) Do COPD patients deficient in 25-hydroxyvitamin D have reduced outdoor activity or altered susceptibility to HRV (including viral load) at exacerbation than those with insufficient or normal levels?
(c) Do VDR polymorphisms differ between frequent and infrequent exacerbators?
The primary outcome measure was the first question and this has now been clarified in the text.

2. Are the methods appropriate and well described?
The processes of patient recruitment to the London COPD cohort are well described and widely accepted as robust. Ethical approval is stated, and definition of exacerbation frequency is both clearly stated and the categories widely accepted. The methods of vitamin D measurement, viral typing and genotyping are adequately described. It is not clear in the methods why these particular VDR polymorphisms were chosen – I presume it is because of known functionality, since association with infectious disease for VDR is mentioned in the discussion. Statistical analysis is described in an appropriate amount of detail, and power stated for the primary outcome measure. The genetic analyses are almost certainly underpowered; this is not stated or discussed. The reason for choosing these polymorphisms is as above and this has been clarified in the text. The underpowered genetic analysis is discussed.

3. Are the data sound?
Sufficient detail on the patient cohort is given in either text or tables in the results section. The data shows the expected change in vitamin D levels between seasons in patients who were not taking calcichew D3, and that this difference was not detectable in the patients on this supplement. There was no difference in
vitamin D levels between frequent and infrequent exacerbators after taking season into account. The expected changes with vitamin D levels with time outdoors were shown (i.e. vitamin D levels dropped with lower sunlight exposure – consistent with known vitamin D biology). There was no relationship between vitamin D, HRV or exacerbation severity, nor were there any significant genetic effects seen. The data seems sound in that it agrees with known vitamin D biology, and the methods for obtaining and analysing it were satisfactory. This does not remove the question of power for secondary outcome measures.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
Yes, the authors discuss their primary outcome measure in context of the data. Concepts pertaining to time spent outdoors and vitamin D levels, and how this might influence management in future are also covered, whilst acknowledging limitations to the conclusions they can make. Similarly the interesting difference between Calcichew D3 supplemented and non-supplemented individuals is discussed in light of current thinking on the amount of D3 required to maintain levels, and small cohort size. Conclusions about the relationship of viral and genetic data to vitamin D are appropriately cautious, since power is uncertain here. I would have welcomed a bit further discussion on the relative influence of bacterial exacerbations, if culture data is available. Unfortunately bacterial culture data is not available but this has now been added to the discussion. It is also important to note that a trial of vitamin D supplementation in COPD has now been published – it would be helpful if these could be added and discussed (Lehouck et al, Ann Intern Med (2012) 156: 105-14). Other data on relationship of vitamin D levels to exacerbations in COPD has been published which would also be useful to add and discuss briefly (Kunisaki et al, AJRCCM (2012) 185: 286-90). Both of these references have been added and discussed.

6. Are limitations of the work clearly stated?
For the most part, yes. Power on secondary outcomes is not usually stated in any manuscript, but some acknowledgment of small cohort size for epidemiological and genetic work could be added to the discussion. Acknowledgement of the small cohort size has been added to the discussion.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes – there are many publications on the London cohort, which are mentioned here.

8. Do the title and abstract accurately convey what has been found?
Yes

9. Is the writing acceptable?
Yes.

Discretionary Revisions
Discussion of power issues for secondary analyses. This has now been done.

Minor Essential Revisions
Confirmation of primary outcome measure in background section. This has been done.

Addition of any data available on bacterial influences on exacerbations in the cohort, and relationship to vitamin D (as done for HRV) – if not available then
lack of data could be acknowledged in discussion. *Lack of data has been discussed.*

Addition of reference to vitamin D trial published in COPD, and their findings in context of those reported by the authors here. Addition of Kunisaki et al, AJRCCM 2012. *The references have been added and discussed.*

Major Compulsory Revisions

Nil

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare I have no competing interests

Yours sincerely,

Jennifer Quint