Author's response to reviews

Title: Pseudomonas aeruginosa isolates in severe Chronic Obstructive Pulmonary Disease: characterization and risk factors.

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Author's response to reviews: see over
Dear Editor,

Please find attached the revised version of our study “Characterization of *Pseudomonas aeruginosa* isolates in severe Chronic Obstructive Pulmonary Disease: the role of bronchiectasis” for consideration and possible publication in your Journal. The final version has been approved by all the co-authors.

We have made the changes proposed by reviewers:

**Reviewer: Marc Miravitlles**

**Line 43. In should be specified at what time point were the 41 isolations of PA obtained**

*P. aeruginosa* was recovered once or more from sputum samples in 41 patients. In five of them, isolates were reported during the year before inclusion with posterior negative samples for this PPM during the follow-up period; twenty-two had isolates growing *P. aeruginosa* before and during the study period, and in the remaining 14 patients, this PPM was recovered only during follow-up. These details on the time point of isolation of *P. aeruginosa* have been clarified in the main body of the text, in the results section.

**Line 50. “in this subset…” which subset are the authors referring to?**

The phrase refers to the subgroup of patients showing chronic colonization by *P. aeruginosa*. The abstract section has been modified to clarify this point.

**Line 111. It is not clear why the authors excluded patients with known bronchiectasis from the study. It does not make much sense to exclude patients with known bronchiectasis from a study aimed to investigate their importance.**

Patients with a primary diagnosis of bronchiectasis, based on reported symptoms and chest X-rays were excluded from this study, independently of their lung function. We consider that patients with this clinical pattern conform a specific population, that has in common the antecedent of severe bronchial disease, and has clear-cut differences with COPD. After a primary diagnosis of COPD, the severity of bronchiectasis which are diagnosed only on CT scans is lower, and manifest in most cases only as chronic bronchitis. The impact of this bronchial abnormality in severe COPD has not been adequately addressed till now, and its relationship with chronic colonization and exacerbations is poorly known, in spite of their frequency. Accordingly, we consider that a study focusing on this clinical situation is needed, and this was the reason to select a population sample of COPD patients with an accurate description of bronchiectasis extension for the present study.
Line 120. The inclusión of patients with positive PA isolation the previous year is a posible source of bias. The authors should verify that the non-PA group also had sputum samples taken the previous year that were negative for PA.

*COPD patients attending the Respiratory Day Care Unit have been visited by the clinical team from 1-3 years before their inclusion in the study. Collection of sputum samples for culture in part of the usual care in the Unit, and all patients have had a minimum of four previous cultures obtained during the two previous years. We have checked accurately the sputum cultures obtained the previous year, to avoid misclassification. The analysis of the results of sputum culture the two previous years did not modified the obtained results and were not included in the revised version of the manuscript, to simplify the presentation of data.*

Line 259. It is surprising the low number of sputum samples obtained in stable phase. This may have resulted in a very low incidence of PA colonisation, just because the number of samples was small. This has to be acknowledged as a limitation of the study. It is also remarkable that some 200 sputum samples were culture negative. This is in relationship with very strict criteria for colonisation (line 296)

We agree with the reviewer that is a limitation and true prevalence of colonization may be underestimated. The study was designed to register exacerbations and for collection of stability samples during the scheduled visits. A significant number of these visits were coincidental with an exacerbation or the immediate weeks following that episode, and, accordingly, can not be considered stability samples. Our results reflect the limited possibilities to recover sputum samples in stable participants in a cohort of severe COPD patients characterized by their frequent exacerbations. The corresponding paragraph has been rewritten to clarify these points.

Line 291. The number of courses of antibiotic was a factor significant in multivariate analysis, but it is not specified in which period of time.

*To clarify the point raised by the reviewer, in the multivariate analysis we have included the variable antibiotic courses/year as a variable that take into account cumulated use of antibiotics. We consider that this approach improves the interpretation of the results.*
Reviewer: Miguel Angel A Martinez-Garcia

1. The exclusion criteria included patients with prior bronchiectasis. If the authors included patients with severe COPD, I am sure that some of them had previous TCAR with bronchiectasis and then they were excluded from the study. I think that this situation biased the study.

For this study, patients with a primary diagnosis of bronchiectasis, based on reported symptoms and chest X-rays were excluded, independently of their lung function. We consider that patients with this clinical pattern conform a specific population, that has in common the antecedent of severe bronchial disease, and has clear-cut differences with COPD. After a primary diagnosis of COPD, the clinical severity of bronchiectasis which are diagnosed only on CT scans is less severe, manifesting in most cases only as chronic bronchitis. The impact of this bronchial abnormality in severe COPD has not been adequately addressed till now, and its relationship with chronic colonization and exacerbations is poorly known, in spite of their frequency. Accordingly, we consider that a study focusing on this clinical situation is needed, and this was the reason to select a population sample of COPD patients, without a previous diagnosis of bronchiectasis, who have an accurate description of bronchiectasis extension in the CT scan performed for the present study.

2. Why the use of macrolides is a exclusion criteria?. I think this is another bias. Severe COPD patients using macrolides probably have more probability of having an isolation by PA and bronchiectasis.

Previous use of antibiotherapy is a risk factor for P. aeruginosa infection, according to current guidelines (Woodhead), and to avoid the influence of chronic use of antibiotics on the bacterial flora of COPD patients we excluded patients following chronic antibiotic therapy (oral or inhaled). We consider that the inclusion of these patients may confound the analysis of the relationships between previous antibiotic courses and the assessed effects.

3. Quantitative measure of CFU?

For this study the result of sputum culture was expressed as a qualitative variable, and bacterial loads were not used as a independent variable, and only to identify patients with positive cultures for P. aeruginosa (>100 cfu/mL). Additional details on the methodology of microbiology processing have been included in the revised version of the manuscript.

4. Were the patients trained to go to the medical consult when they experience an exacerbation?

Severe COPD patients in the cohort attending the Respiratory Day Care Unit of the center follow an education program when entering their scheduled follow-up
protocol, and were provided of a personal self-management plan that included unscheduled visits to the Unit when exacerbation symptoms appear. This approach has been detailed in the methods section of the revised version of the manuscript.

5. Multivariate analysis should be better defined in the statistical section in terms of included variables and adjusted variables. Why do you use enter method?. I think a table with the results of this analysis should be added to the paper.

We have followed the recommendation of the reviewer and the revised version of the manuscript has been modified accordingly. Variables significant in the model (p<0.10) were selected for the multivariate analysis, excluding those clearly collinear (such antibiotics courses and antibiotics/year), and were entered in the model in a step.

6. Are there differences between patients with isolation of PA in the previous year, during the exacerbation and during the follow-up?.

The cohort was very homogeneous; differences between the three groups were analyzed and not differences in antibiotics prescriptions or extent of bronchiectasis were found (data not shown).

7. How many patients without sputum samples? ¿What is the median of sputa per patient?.

All patients had sputum samples, although in some patients the number of sputum samples was higher due to a higher exacerbation frequency. The observed median and interquartil range for sputum samples available was 3 (1-6), reflecting the high number of sputum samples available in most patients.

8. The authors think that those patients with an unique isolation in the previous year with PA eradication should be included in the PA group?.

It is not uncommon that P. aeruginosa strains chronically colonizing the bronchial tree appear repeatedly in sputum cultures, sometimes with negative cultures for that microorganism between positive cultures, as has been demonstrated in previous cohort studies, that have demonstrated the persistence of the same strain through pulse-field electroforesis, combined with PCR positive results from cultures appearing as negative. Accordingly, we considered both positive cultures for P. aeruginosa at baseline and/or the previous year as equivalent, independently of the results of culture in the follow-up year.

9. Was there multicollineality between the independent variables in the logistic regression?. For example between the presence of bronchiectasis and the number of antibiotics?.
Multicolineality was tested and not present between the covariates included in the final model. We evaluated multicollinearity by Variance Inflation Factor (VIF) and test of tolerance and the results have been considered to be negative. The VIF measures the impact of collinearity among the variables in a regression model and was below 2.5 for each covariate in our model.

10. I think this study can “suggest” but never “demonstrated” (see conclusions)

We agree with the comment of the reviewer and we have modified the revised version of the manuscript accordingly.

Minor Comments

1. Title. Why the role of bronchiectasis and not the role of previous antibiotics?. I think that “the role of bronchiectasis” should be removed from the title.

When we designed the study we knew that FEV1 was an important determinant of P. aeruginosa isolation, but in clinical practice the majority of severe COPD patients do not show P aeruginosa isolates, therefore, we hypotetized that non-diagnosed bronchiectasis may play a role in the appearance of positive sputum cultures for this PPM and chronic colonization. Our results show that, apart from the extent of bronchiectasis, the frequent use of antibiotics is also an independent predictor of P aeruginosa isolation. The title has been changed accordingly, following the recommendation of the reviewer.

2. I think that the isolation percentage of P aeruginosa in the studied sample is very high (40%).

We agree that the prevalence of P. aeruginosa in the studied sample is high, but we consider that this is attributable to the selection for the study of severe COPD patients reporting frequent exacerbations in the previous year, who are currently cathegorized as type D according with GOLD criteria. This population sample have specific characteristics that differentiate them inside the whole population of COPD patients, and determine specific colonization and infection patterns. The severity of these patients and their higher need of care justify focusing on this population, with the assumption that results obtained on them are not extrapolable to the COPD population as a whole.

3. Have chronic colonization and chronic infection the same definition in the opinion of the authors?.

The only difference between chronic colonization and chronic infection are the presence of respiratory symptoms. In severe COPD patients with frequent exacerbations bronchial secretion cultures appearing repeatedly positive for a PPM often appear both in stability and exacerbation periods, and the discrimination between chronic colonization and chronic infection in these
patients is unclear. Accordingly, we consider that chronic colonization as a unique term may be used for the description of this clinical situation. Considering that the chronic recovery of P. aeruginosa from a COPD has the implication that the microorganism stays in the bronchial tree during the stable periods, we have selected the term chronic colonization as the descriptor of this situation in the revised version of the manuscript. Details on the definition of the term have been included in the methods section.

4. Antibiotic treatment was prescribed in accordance with their hospital clinical practice guidelines. Are these guidelines different from the national or international guidelines?

We use the same antibiotics that those recommended international (included in the revised manuscript), adjusting empiric treatment accordingly to the antibiogram when available.

1. I think some scores such as Bhalla score would is desirable to better characterized the severity of bronchiectasis.

Different scores have been proposed to be used for the description of bronchiectasis on CT scans. In the study we have followed a validated approach for bronchiectasis scoring, which has been widely used and has the advantage of its simplicity. We consider this approach as appropriate for the purposes of the study, and we think that the inclusion of an additional score will not add value to the obtained results.

2. I think the authors should stated if they think that a HRCT scan should be performed in all severe COPD patients with or without exacerbations or in those patients with multiple antibiotic use in accordance with their results. Do the authors think that in the clinical practice it is important to perform some microbiological sputum analysis during the exacerbations in severe COPD patients?

As suggested by the reviewer, and according with the results of the present study, an extended study of severe COPD patients with frequent exacerbations may be recommended, which needs to include CT scans and repeated bronchial secretion cultures. The severity of this population justifies this approach, and the results of the proposed explorations in these patients will give useful information for prognosis and treatment adjustments. This approach, however, may not be extrapolated to the COPD population as a whole. This comment has been included in the discussion section of the current version of the manuscript.
Thank you in advance for your consideration and we look forward to hearing from you at your convenience.

Miguel Gallego, MD, on behalf of the authors
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