Author's response to reviews

Title: Role of C-reactive protein as a biomarker for prediction of the severity of pulmonary exacerbations in patients with cystic fibrosis.

Authors:

Rosa María Girón Moreno (rmgiron@gmail.com)
Jose Luis Justicia (jljusticia@gmail.com)
Sara Yamamoto (saiux_25@hotmail.com)
Claudia Valenzuela (claudiavale@hotmail.com)
Carolina Cisneros (carol9199@yahoo.es)
Rosa Mar Gómez-Punter (rosamar_gp@hotmail.com)
Gilda Fernandes (gmfdes@hotmail.com)
Julio Ancochea (juli119@gmail.com)

Version: 3 Date: 13 August 2014

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

Below you will find the response to referees’ comments regarding the following manuscript:

MS: 2735005731296060
Research article
Role of C-reactive protein as a biomarker for prediction of the severity of pulmonary exacerbations in patients with cystic fibrosis.
Rosa María Girón Moreno, Jose Luis Justicia, Sara Yamamoto, Claudia Valenzuela, Carolina Cisneros, Rosa Mar Gómez-Punter, Gilda Fernandes and Julio Ancochea.
BMC Pulmonary Medicine

Reviewer 1

Major Compulsory Revisions

1. Exacerbation severity index:
   - Item b) – this index is not practical to apply clinically as it requires the final FEV1 which is not available at the time of admission
   The exacerbation severity index is not a predictive index but a tool for estimating the severity of a pulmonary exacerbation based on 4 parameters that can be only obtained retrospectively after the patient has suffered the exacerbation: loss of lung function after an exacerbation, recovery of lung function after an exacerbation, number of days receiving IV antibiotics in hospital, and complications; none of them can be achieved at the time of admission.
   - Item c) - why was this only based on the number of days requiring IV antibiotics in hospital. What about days of IV antibiotics at home?
   Number of days receiving IV antibiotics in hospital was chosen as one of the items instead of total number of days of IV antibiotics (including antibiotics at home) because the first one reflects more appropriately the severity of an exacerbation. Many times in clinical practice, patients are discharged from hospital but they keep routinely taking medicines for some days in spite of the fact that their exacerbation is considered resolved. This last sentence has been added to the section “Pulmonary exacerbation severity index”.
   - What about including sputum microbiology such as BCC or MDR-PA?
   Including sputum microbiology such as BCC or MDR-PA, both related with worse prognosis of CF patients, might be a good predictor of severity of exacerbations, but this wa out of the scope of our study. In addition, only 3 and 7 patients were colonized by BCC and MDR-PA respectively, so it is unlikely that including microbiology in the severity index would have added some relevant information.

2. It is unclear if each of the 62 exacerbations from 27 unique patients analyzed as separate events? If yes, did the analysis factor in the correlated data as some patients had multiple exacerbations? Consider analyzing just 1 exacerbation per patient. For table 2, the number of exacerbation events should be included.
   Each of the 62 exacerbations was analyzed as separate event. The number of exacerbations has been included in the table 2 and the following sentence has been added to the section “Statistical analysis”: “When a patient suffered multiple pulmonary exacerbations along the study period, each exacerbation was analyzed as a separate event”. Although most patients had several exacerbations, these had different
characteristics, including values of CRP before each unique exacerbation (e.g. the same patient may first show a moderate exacerbation with CRP = 2.4 mg/dL and later they may suffer a very severe exacerbation with CRP = 5.9 mg/dL). That’s why we didn’t consider analyzing just 1 exacerbation per patient.

3. What percentage of patients had oral antibiotics prior to admission and initiation of IV antibiotics? This may influence the admission CRP measurements and mask the relationship between CRP and exacerbation severity.

Prior to admission, 25 (40.3%) exacerbations were treated with oral antibiotics (19.4% with quinolones, 9.7% with amoxicillin, 8.1% with co-trimoxazole and 3.2% with others) for 3 to 7 days in 9 (14.5%) cases, 8 to 14 days in 11 (17.7%), and 21 days in 5 (8.1%). This sentence has been added to the second paragraph of “Results”.

Mean values of CRP were similar in patients previously treated (4.2 mg/dL) or not treated (3.8 mg/dL) with oral antibiotics (p=0.405; Mann-Whitney test). Duration of treatment didn’t seem to influence the CRP values either: 4.8 mg/dL, 3.6 mg/dL, and 4.4 mg/dL in patients treated for 3 to 7 days, 8 to 14 days, and 21 days, respectively (p=0.823; Kruskal-Wallis test). These results have been added before the last paragraph of “Results”.

4. What is known about changes in CRP within the first 48 hours of treatment? If the half-life is short and the patient is responding to treatment, CRP levels might fall quickly and the CRP value might have less ability to predict exacerbation severity if the measurement was delayed. A sensitivity analysis looking at the subgroup with CRP measurements within 24 hours should be considered.

A sensitivity analysis of the association between CRP level and the severity index has been performed selecting the exacerbations with CRP measurements within 24 hours after hospital admission (n=31 [50%]). Using Spearman’s rank correlation test, no relationship between the two variables was observed (p=0.344). This text and the reviewer’s comments have been added to “Statistical analysis”, “Results” and “Discussion”. A new reference (#34) has been added to “References”.

5. How was CRP measured? hsCRP or not? Clinical or research lab? These details should be provided in the Methods.

CRP was measured by immunoturbidimetric assay (Tina-quant CRP detection method; Roche Diagnostics) at our hospital clinical laboratory. This has been added to the section “Methods”.

6. Page 8, line 187: How was larger number of exacerbations defined? Why the focus just on p-values for this comparison.

As opposite to the rest of baseline patients’ characteristics associated to CRP, the number of exacerbations treated with IV antibiotics during the previous year was considered as a quantitative variable. Using Spearman correlation method, statistically significant association (p=0.001) between number of exacerbations treated with IV antibiotics and CRP (both quantitative variables) was shown. That means that the higher the number of exacerbations, the higher the value of CRP. In this case, only focusing on p-value is possible.

7. Multivariable analysis should be conducted in the analysis of the association between CRP and baseline characteristics to identify independent baseline factors associated with CRP.

Multivariable analysis with baseline characteristics showing statistically significant (p<0.05) association with CRP (colonization by P. aeruginosa, ABPA, treatment with oral corticosteroids and number of exacerbations treated with IV antibiotics during the previous year) was conducted. By linear regression, only treatment with oral
corticosteroids was identified as an independent factor associated with CRP values (beta coefficient = 3.012; p=0.005). This has been added to “Statistical analysis” and “Results”.

Minor Essential Revisions

1. Page 7, line 166: Initial CRP values were associated with FEV1 – which FEV1 measurement does this refer to? Admission or baseline?
   It refers to baseline FEV1. Text corrected in that line.

2. What were the indications for oral corticosteroid use and were the patients started on this therapy prior to CRP measurement? Were these the same individuals that had ABPA? Multivariable analysis would be useful as mentioned above.
   18 episodes of exacerbations had been receiving continuous treatment with oral corticosteroids during the 3 months before the episode begins. In 17 (94.4%) there was a diagnosis of ABPA, so it seems that those are the same individuals. In order to clarify this relationship, a multivariable analysis has been carried out.

3. Exacerbation severity index – as the authors have acknowledged, this composite score has not been validated and therefore the scoring system might not be comprehensive enough to assess exacerbation severity. This should be mentioned in the limitations.
   This comment has been added to the paragraph where limitations are mentioned.
Reviewer 2

Major Compulsory Revisions:

1. The authors report an association between CRP levels and P. aeruginosa and aspergillosis. However, they do not report their findings regarding other bacteria which were colonising the patients, in particular, the 66.7% who were colonized with S. aureus. The authors quote Watkin et al as finding lower CRP in patients with S. aureus – did the authors of this study find the same lower levels? The sentence “Association between CRP and the rest of baseline characteristics was not statistically significant” has been added to the last paragraph before the section “Discussion”. A multivariable analysis has been conducted and the findings have been added to “Results” and “Discussion”.

2. There is a poor representation of data in the results section and better use could be made of results – basically findings are summarised into one table. There should be other graphical representations to compare results before and after exacerbation in chart form and also correlation graphs between several of the parameters. Figure 1 showing the FEV1 values before and after the exacerbations, and Figure 2 showing the correlation between CRP and the severity index have been added to Results.

3. Correlations should be made between CRP and other inflammatory biomarkers such as IL-8 and HNE. The authors present values for IgG, IgA and IgM – were these total titre values – if so, then these are non-specific or where they against P. aeruginosa antigens for example? IL-8 and NE were not measured. IgG, IgA and IgM refer to total non-specific titre values. This clarification has been added to Table 2.

Recommendations for Improvement:

1. The discussion section should contain more specific information with regards to correlation of CRP to well documented specific pulmonary biomarkers such as IL-8 and NE. There is little mention to the inflammatory biomarker values reported in table 2. Was there any correlation with the antibody titres and other inflammatory markers with the severity index? Correlation tests between the severity index and other inflammatory markers (leukocytes, ESR, IgG, IgA, IgM and fibrinogen) have been done but significant association has not been found. The text “No correlation between the severity index and other inflammatory markers (leukocytes, ESR, IgG, IgA, IgM and fibrinogen) was found” has been added to Results.

2. Neutrophil Elastase mentioned in the discussion is an important biomarker in detecting and monitoring pulmonary exacerbations. This biomarker has been found to correlate with CRP therefore, was this measured in the study? Of particular note, this biomarker may be more relevant to the severity index rather than CRP. According the reviewer’s comment, the following sentence has been added when the limitations of the study are highlighted in the “Discussion” section: “For example, neutrophil elastase and interleukin-8 are important biomarkers in detecting and monitoring pulmonary exacerbations (16,38) so they may be more relevant to the severity index than CRP. A new reference (#38: Martin SL et al) has been added.
OTHER CORRECTIONS

Error in page 8, line 188, has been detected and corrected: mean value of CRP in patients taking oral corticosteroids was 6.1 and not 4.7

Reference #16 has been updated.