Author’s response to reviews

Title: C-reactive protein serum levels as an early predictor of outcome in patients with pandemic H1N1 influenza A virus infection

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Version: 3 Date: 13 July 2010

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

We were pleased to hear that our manuscript titled: “C-reactive protein serum levels as an early predictor of outcome in patients with pandemic H1N1 influenza A virus infection” is now considered for publication.

We have performed all the necessary changes as suggested by the reviewers. Below is a list of the reviewer comments with our answers written in red. Attached please find our revised manuscript, where you will note that all changes have been entered using the Track Changes tool.

We hope that you will find our changes satisfactory and that our manuscript will be considered suitable for publication.

Sincerely,

Ofer Zimmerman, M.D.
**Associate Editor's comments:**

The reviewers have raised numerous comments related to the study design, how results were reported, and the discussion. I would encourage the authors to reply carefully to these comments. Particularly, the authors should discuss more extensively the limitations and bias resulting from the retrospective design and exclusion of 150 patients.

*As suggested, the discussion was expanded to better explain the limitations of the current study.*

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

*We have addressed point by point all of the reviewers’ comments.*

Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals ). It is important that your files are correctly formatted.

*We have highlighted (with Tracked Changes) all changes made in the manuscript.*

The changes were made to match the journal style.

Further consideration of your manuscript is conditional on improvement of the English used. Please ensure particular attention is paid to the abstract. You should
have a native English speaking colleague help you with this, if possible, or use a commercial copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

The manuscript in general and the abstract in particular were edited by a professional native English speaker editor.

In addition, please can you state the name of the institutional review board that provided ethical approval for your study. Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

The name of the institutional review board was added to the Method section.

The abstract also needs to be revised according to our guidelines (http://www.biomedcentral.com/info/ifora/abstracts). The abstracts of manuscripts submitted to the BMC-series should be structured as follows:
- **Background:** This should place the study into the context of the current knowledge in its field and list the purpose of the work; in other words, the authors should summarise why they carried out their research.

- **Methods:** This should summarize how the study was performed and mention the different techniques employed. It should also include details of any statistical tests employed.

- **Results:** This section should describe the main findings of the study.

- **Conclusions:** A brief summary of the content of the manuscript and the potential implications of its results.

The abstract was revised in accordance with BMC medical journals guidelines.

**Reviewer:** Charles-Edouard Luyt

**Major compulsory revisions:**

1. The main limitation of this study, as for every study on a diagnostic or a prognostic marker, is the meaning at the patient level. The authors found that mean CRP level was higher in patients with subsequent ICU admission or mechanical ventilation than in patients with uncomplicated course. However, there is an overlap in the values (Figures 1 and 2). Thus, for a given patient, a value of CRP does not mean something.

We believe that our findings are significant also at the individual patient level. Although there is an overlap in the CRP level, serum CRP levels constituted the best predictor of subsequent patient ICU care and the only predictor of subsequent mechanical ventilation. Moreover, an initial low serum CRP level was an excellent predictor of good outcome: none of the patients with CRP levels lower than 33 mg/L required either ICU care or mechanical ventilation.
The fact that patients with low serum CRP levels enjoyed good outcome may help the assessing clinician at the ED to differentiate between "low risk" and "high risk" patients. We have changed the discussion of the manuscript and accentuated the clinical relevance of CRP measurement at the individual patient level (please see page 12, last paragraph).

2. The best way to express the results for a diagnostic test is probably to give the positive and negative likelihood ratios. If I am correct, the corresponding values for CRP to predict ICU admission are 2.6 and 0.5, respectively. These values are not typical of a good test.

Although likelihood ratio is an important parameter for a diagnostic test, it does not tell the whole story. In the present manuscript, CRP is not presented as a diagnostic test for H1N1 infection, but as an aiding tool for identifying patients at low risk for severe prognosis. Hence, we believe that the high sensitivity and negative predictive value are important and should be evaluated and taken into consideration by the clinician that considers the best approach (mainly hospitalization vs. discharge) for a patient in the ER.

3. As underlined by the authors, this study is retrospective, and 40% of the 315 patients were not evaluated. Thus, it is difficult to draw definite conclusions and I suggest them to be more cautious in the interpretation of their results. As an example, in the conclusion of the abstract, the authors should specify that these results are true for their population.
The abstract and discussion were changed in accordance with the comments (Please see Abstract page 3, last paragraph, Discussion, page 14, second paragraph).

4. The authors should discuss their limitations more extensively. For example, it is not sure that the results can be extrapolated to another population.

The discussion was revised to better explain the limitations of the current study (Please see Discussion, page 14, second paragraph).

5. The authors chose a model to show that CRP was an independent factor associated with ICU admission and mechanical ventilation. However, these parameters were obtained on the emergency department. Since most patients first stayed in the ED and were secondly referred to the ICU or the ward, it would have been more interesting to look at the kinetic of those parameters.

As stated above (comment 1 and 2), CRP does not seem to be a good test to predict ICU transfer.

We agree with the reviewer that CRP kinetics would probably better reflect the overall patient outcome. However, the scope of this research study was to identify prognostic factors among patients with H1N1 virus infection presented to the emergency room.

6. The authors stated that CRP was the best predictor of whether a patient would need ICU care and mechanical ventilation. Again, this is true only when looking at the parameters obtained on ED admission, with the model the
authors chose. The authors have to underline this point because it can be confusing for the reader.

The scope of the current manuscript was to evaluate the parameters obtained on ED admission. In order to better underline this point to the reader, we added it to the last paragraph of the introduction (page 5), and in the conclusion (page 14-5).

7. More interesting for a clinician is the course of the disease. Do the authors have any data? How was the clinical course? And the evolution of blood gazes?

The clinical course of most of the patients was unremarkable – as only 17 (9%) patients were admitted to ICU, eight (4%) required mechanical ventilation and three (2%) died. In a telephonic follow-up survey, we located 185 (98%) of the 188 survivors. In regard to blood gases, since this data was available for a minority of the patients, we unfortunately couldn’t relate to this parameter or evaluate its relevance to the prognosis and clinical course of the disease. We have added a comment on this matter in the Results section (page 10, second paragraph).

8. If I correctly understand what the authors did in there model was a stepwise forward analysis (this is not underlined in the methods. Please add the methodology in the corresponding section). What are the results if the authors enter first the results of the blood gazes (PaO2), then chest X-Ray and then abnormal auscultatory findings? Does CRP level entered into the model?
We have added the method of the Cox regression model to the statistical analysis section (page 8). As suggested, using the steps described, low saturation was entered first. The chest X-Ray did not add any significant data, but the abnormal auscultatory findings did. At this point, CRP did add valuable information to the model in addition to low PaO2 and abnormal auscultatory findings. Since those steps did not change the results significantly, we did not include it in the manuscript.

**Reviewer: xiao hong**

Method: In method section, the design of the study, the setting should be presented and this study was conducted to examine the role of CRP level as a early predictor for prognosis, however it not clear what were the methods used to assess this.

We have added some data regarding the setting of the study and added clarification to the statistical method section (please see Materials and Methods section, pages 6-8)

Results: The result section is not well reflecting the results of the study, the authors should add the cut-off value of CRP by ROC curve analysis, and its specificity and sensitivity in predicting the sever outcome, so this section should modified accordingly.

As suggested, we now included an additional table (Table 2, page 22) with several cut-off values and their respective sensitivity, specificity and PPV and NPV for the
different outcomes and changed the results section accordingly (page 11, last paragraph).

Study design: The author should make it clear that under which criteria the patients should be admitted to ICU. This is very important for us to understand the value of serum CRP level as a predictor for severe outcome, and the quality of a ROC curve analysis, depends largely on the inclusion criteria of the patients with influenza A(H1N1) to ICU.

The criteria in our hospital to transfer patients to the ICU are determined on a clinical basis. In our hospital, patients with H1N1 infection were transferred to the ICU if they developed respiratory failure.