Author's response to reviews

Title: Measurement of complement receptor 1 on neutrophils in bacterial and viral pneumonia

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Author's response to reviews: see over
Dear Sirs/Madams,

Thank you very much for your positive letter regarding our manuscript “Measurement of complement receptor 1 on neutrophils in bacterial and viral pneumonia” by doctors Ulla Hohenthal, Jari Nuutila, Esa-Matti Lilius, Iina Laitinen, Jukka Nikoskelainen and myself. We were pleased to receive a request of a revised version of this manuscript. We found the criticism and comments presented during the reviewing process constructive, and feel that they have helped us to improve the manuscript. The manuscript has been revised according to these suggestions.

We include here a listed response to all of the comments presented by the Reviewers. We hope that after these revisions and clarifications, you will find this information suitable to be published in your Journal. Finally, we would like to thank you and the Reviewers for your time, consideration and valuable criticism.

Yours sincerely,

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Comments on the criticism presented by the Reviewers and a list of the changes and additions made according to their suggestions.

Reviewer #1 (Chris Garrard)

General

We thank the Reviewer for his kind words regarding the interest of our work.

The Reviewer notes that we focus upon the neutrophil CR1 expression in pneumococcal pneumonia as our key finding and feels that the multi-component response of CRP, ESR, and several cell receptors could possibly be discussed in the manuscript. Indeed, we totally agree with the Reviewer in this point. After these preliminary assays, we have already started to evaluate the clinical usefulness of a combination of these parameters in a larger patient population. Following his advise, we have added a new chapter in the end of the Discussion section (page 9, lines 1-6) focusing on this issue as follows:

“Although the high expression of neutrophil CR1 is suggestive of classical bacterial pneumonia, it is unlikely that any single parameter of inflammation alone could reliably differentiate between bacterial and viral pneumonia. Rather, it is possible that the diagnostic accuracy could be improved by combination of the results of CRP, ESR, and several cell receptors. Studies are presently underway to determine, whether the diagnostic yield provided by the measured individual variables would increase upon combination.”

In his General remarks, the Reviewer quite correctly assumes that for this preliminary report, efforts were not made to identify the time period between the onset of the symptoms of pneumonia and the time point of the receptor analysis.

The Reviewer notes that no CRP or ESR data for controls are given in the Table, and correctly assumes that these values were not analysed for the controls. This information has been added in the text (page 5, line 21).

Major Compulsory Revisions

1. The Reviewer asks, who established the diagnosis of pneumonia and how was this done.

In the patients included in this study, the diagnosis of pneumonia was based on the presence of an infiltrate on chest radiograph in association with fever and/or respiratory symptoms for which no other cause was found.
During the study period, both Ulla Hohenthal and Pirkko Kotilainen were working in the Infectious Diseases Unit of the Turku University Hospital, UH as an Infectious Diseases Specialist and Ward Physician and Pirkko Kotilainen as the Chief Medical Officer of that Unit. The diagnosis of pneumonia was made by these 2 authors by consensus. In addition, the chest X rays were examined by a specialist in radiology.

Data on the establishment of the diagnosis of pneumonia are presented in the text (page 4, lines 2-6, revised manuscript)

2. The Reviewer asks, whether the authors can provide some discussion on the relative timelines of the various acute phase proteins and receptor responses.

The answer is that the results of the analyses of various acute phase proteins and leukocyte receptor responses used in comparisons were always performed from the blood samples taken at the same time. This means that a patient may have had a CRP value and WBC count analysed already on admission, but these parameters were not used in the comparisons. After the patient had given a written consent, blood samples were collected simultaneously for both the receptor analyses and the determinations of the CRP, ESR, and WBC. This took place usually on the next morning and always within 2 days from the admission.

This information was included in the original manuscript, but maybe not clearly enough. Therefore, we have made efforts to describe these timelines more clearly in the revised manuscript (Page 5, lines 7-8 and 17-19) as follows:

"For the measurement of leukocyte receptor expression, 10 ml of heparin anticoagulated blood was collected from the patients within 2 days of hospital admission."

"Concurrently with the collection of blood for the measurement of leukocyte receptor expression, blood or plasma samples were taken for the measurement of CRP, erythrocyte sedimentation rate (ESR), and white blood cell count (WBC)."

3. The Reviewer asks, whether all patients included were competent to provide a written consent, i.e. none was mechanically ventilated and required sedation.

The answer is that all were competent. None of the patients needed mechanical ventilation or treatment in the intensive care unit. This information has been added in the text (page 4, line 10-11, revised manuscript).

4. The Reviewer comments on that the statistical analyses were based on multiple t-tests and asks whether correction for multiple comparisons was made. He also recommends that an experienced statistician would be consulted on whether the statistical approach was appropriate.

Following his advise, we have asked Dr. Hans Helenius, Msc, to help us with the statistical analyses. Dr. Helenius is an experienced statistician serving as a senior lecturer at the Department of Biostatistics, University of Turku, Turku, Finland.
Admittedly, Dr. Helenius was not quite happy with the statistical methods we originally used in this work. Therefore, the data presented in the text and the Table have been re-examined using the statistical methods preferred by Dr. Helenius. First, group differences between the groups A (pneumococcal pneumonia), B (influenza A pneumonia), C (aetiologically undefined pneumonia, and D (controls)) were tested using analysis of variance (ANOVA). Pairwise, group comparisons after ANOVA were carried out using Tukey’s multiple comparison technique. This technique is optimal in the case when all pairwise comparisons of the groups are carried out.

These new methods have been described in the text (page 5, lines 23-25, revised manuscript).

The results provided by the above mentioned statistical methods remained practically the same as those provided by the methods originally used. The significant differences observed in our original analyses between the groups regarding the different inflammatory parameters remained significant also in these re-analyses. Moreover, the numerical p values remained practically the same. Thus, these re-analyses did not induce any changes in the text except for the total WBC count. As a new finding, total WBC was significantly lower in influenza A pneumonia than in aetiologically undefined pneumonia. Consequently, we have added in the text (page 6, lines 11-14, revised manuscript) the following information:

"Total WBC was significantly lower in influenza A pneumonia than in aetiologically undefined pneumonia, but there was no difference in WBC between influenza A pneumonia and pneumococcal pneumonia."

In the revised version of the manuscript, we now present the more accurate data for the p values provided by Dr. Helenius. First, we have added in the Table the p values for overall group differences (ANOVA) between the groups A, B, C, and D. Second, all p values given for pairwise group comparisons are those provided by Dr. Helenius using Tukey’s multiple comparison technique.

**Minor Essential Revisions**

None required

**Discretionary Revisions**

None required

**Reviewer #2** (Ekkehard Vollmer)

The Reviewer presented no recommendations for revision.