Author's response to reviews

Title: Diagnostic value of triggering receptor expressed on myeloid cells-1 and C-reactive protein for patients with lung infiltrates: an observational study

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

I wish to thank you for the review of our manuscript entitled “Diagnostic value of triggering receptor expressed on myeloid cells-1 and C-reactive protein for patients with lung infiltrates: an observational study”. Please find attached a revised version of our manuscript along the comments raised by the reviewers. Modifications performed are highlighted in the manuscript. You may also find below a point-to-point reply to all comments raised by the reviewers.

We hope that this extensive revision has ended in a manuscript meeting the requirements for publication in *BMC Infectious Diseases*.

Looking forward to your reply,

Best regards,

I. Porfyridis, MD

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**Reviewer 1**

- *First of all, I wonder about the number of authors contributing equally to this work. In detail, all authors except the first author have an equal substantial contributing?*
  
  **Response**: The delegation of responsibilities has now been clearly re-written on pg16, ln13-21 of the revised manuscript.

- *Please add a flow chart of the randomization.*
  
  **Response**: A randomization chart has been added and named Figure 1. Enumeration of the remaining figures has been changed accordingly.

- *How did you calculate the number of patients? Please explain in detail and add the statistical material.*
  
  **Response**: The revised manuscript now reads, on pg8, ln2-4: “Assuming that measured parameters between groups A and B differed by 50%, it was
calculated that 30 to 40 patients should be assigned into each group to yield a difference at the 5% level with 80% power."

- **Background:** Please do not forget controversial and contradictory data of the value of sTREM-1 regarding the predictive role (Tejera et al. Cytokine 2007; 38:117, Dimopolou et al. Inflammation Res 2009;57:1, Bopp et al. EJA 2009; 26,504).

**Response:** The manuscript now reads, on pg3, ln16 to pg4, ln8: “It behaves like a pattern recognition receptor since its activation leads to the release of pro-inflammatory cytokines, namely of tumour necrosis factor-alpha (TNFα) and of interleukin (IL)-8. Although its ligand is still unknown, activation is mediated by bacteria and fungi [3, 4]. A soluble form of TREM-1, namely sTREM-1, is increased in the bronchoalveolar lavage (BAL) of patients with ventilator associated pneumonia (VAP) [5,6], and in the serum of patients with sepsis, with bacterial meningitis and with acute pancreatitis [7-12]. This same soluble form of TREM-1 seems to be increased in patients bearing non-infectious processes like peptic ulcer, inflammatory bowel disease, viral infections, malignant pleural effusions and chronic obstructive pulmonary disease (COPD) but also among patients after cardiac surgery or cardiac arrest. Increase of sTREM-1 seems particular prominent when the latter non-infectious states are complicated with systemic inflammatory response syndrome (SIRS) without infection [13-19]. Several published studies yielded contradictory results for the diagnostic usefulness of TREM-1 and of sTREM-1 for infections [5, 7, 20-22]. The created impression is that more data are necessary to yield definitive results for its usefulness as a diagnostic and prognostic marker of community acquired pneumonia (CAP).”

Several new references have been added, as asked by both reviewers and added in the list of references. Consecutive enumeration of the references in the manuscript has been amended accordingly.
- Laboratory investigation: How many times did you perform your ELISA? 2 or 3 times?
  
  Response: The manuscript now reads, on pg7, ln14: “Determination of sTREM-1 was performed in duplicate by a developmental...”

- Statistical analysis. Why did you choose a cut-off value of 180 pg/ml? This data are from complete different patients with other medical problems. Please explain your transfer a provide substantial data.
  
  Response: The manuscript now reads, on pg8, ln12-15: “This concentration has been proposed as a threshold defining final prognosis in septic populations [22, 29]. Since CAP is a common cause of sepsis, this threshold was considered of merit.

- Results: I am impressed of the 26% mortality in group A. What are the reasons for this high number? What were the comorbidities of these patients? You didn’t mentioned anything about the rate, mode, length or influence of ventilation in your patients. Please add a appropriate section in you paper and state clear these points.
  
  Response: The manuscript now reads, on pg10, ln1-18: “Among patients with group A and CAP nine (n=9) died; six patients were admitted to the ICU and three were not admitted to the ICU due to relatives’ denial. Mean age of patients not admitted to ICU was 80 years old; the first two patients had a case-history of stroke and chronic heart failure; the third patient had a case-history of lung cancer. All three died from severe sepsis and multiorgan dysfunction syndrome (MODS). Mean age of patients admitted to ICU was 70 years old; two patients had a case-history of aortic valve stenosis; two other patients were under chronic intake of receiving corticosteroids; the fifth patient suffered from end-stage renal disease; and the sixth patient was suffering from hepatic failure due to alcohol intake. All six patients died from severe sepsis and multiorgan dysfunction syndrome (MODS). All patients in the ICU accomplished the clinical and radiological criteria for ARDS and were ventilated with the strategy of low tidal volume ventilation, according to current guidelines [30], with volume limited mode ventilation, low tidal volumes (about
6ml/kg ideal body weight), a maximum of 25-30 breaths per minute, high positive end-expiratory pressure PEEP (10cmH₂O) and a goal plateau airway pressure <30 cmH₂O. Among patients admitted in the ICU, two died on the second day post-admission; one died on the third day post-admission; one on the seventh day post-admission; one the eighth day post-admission; and one on the twentieth day post admission.

- **Why did you choose CRP and not PCT as control, or at least additional PCT in your patient management?**
  Response: Although that this reviewer is right about the well proven validity of PCT, even in the studies of PCT evaluation CRP was used as a comparator. This is stated, on pg7, ln19-21 of the manuscript.

- **Did you perform CT scans regularly in respiratory patients with suspect focus? Would I suggest that the CT scan can add relevant informations to distinguish between the proposed 2 groups. Please discuss in detail.**
  Response: The manuscript now reads, on pg6, ln17-18: “All patients assigned to group B were subject to chest computed tomography.”

- **Finally, your English language need a revision in detail to improve you manuscript.**
  Response: The manuscript has been thoroughly copyedited to improve English.
**Reviewer 2**

- The authors state that it is important to differentiate pulmonary infiltrate of bacterial origin from that of non-infectious ethiology, in order to start the appropriate therapy. TREM-1 is a receptor that amplifies the inflammatory response by producing inflammatory mediators, and although there is no recognized ligand until now, some reports propose PAMPs like LPS or DAMPs like Hsp70, as possible TREM-1 ligands. It was reported that TREM-1 ligand could be found in the serum of septic patients (Clin Exp Immunol 2006;145:448). The authors include references to the works of Koussoulas, Tzivras, Ho, Mohamadzadeh and Radsak, which report increased TREM-1 expression related to non-infectious inflammation. Besides them, there are other reports by Adib-Conquy (Shock 2007;28:406) and Isibasi’s group (J Surg Res 2008;150:110, Crit Care 2009;14:R69), both proposing increased TREM-1 expression associated with non-infectious diseases. The last group reported increased expression of TREM-1 in surgical patients with Systemic Inflammatory Response Syndrome (SIRS) and in patients with acute pancreatitis, both since the beginning of their illness.

**Response:** The manuscript now reads, on pg3, ln16 to pg4, ln8: “It behaves like a pattern recognition receptor since its activation leads to the release of pro-inflammatory cytokines, namely of tumour necrosis factor-alpha (TNFα) and of interleukin (IL)-8. Although its ligand is still unknown, activation is mediated by bacteria and fungi [3, 4]. A soluble form of TREM-1, namely sTREM-1, is increased in the bronchoalveolar lavage (BAL) of patients with ventilator associated pneumonia (VAP) [5,6], and in the serum of patients with sepsis, with bacterial meningitis and with acute pancreatitis [7-12]. This same soluble form of TREM-1 seems to be increased in patients bearing non-infectious processes like peptic ulcer, inflammatory bowel disease, viral infections, malignant pleural effusions and chronic obstructive pulmonary disease (COPD) but also among patients after cardiac surgery or cardiac arrest. Increase of sTREM-1 seems particular prominent when the latter non-infectious states are complicated with systemic inflammatory response syndrome (SIRS) without infection [13-19]. Several published studies yielded contradictory results for the diagnostic usefulness of TREM-1 and of sTREM-1.
for infections [5, 7, 20-22]. The created impression is that more data are necessary to yield definitive results for its usefulness as a diagnostic and prognostic marker of community acquired pneumonia (CAP).”

Several new references have been added, as asked by both reviewers and added in the list of references. Consecutive enumeration of the references in the manuscript has been amended accordingly.

• The standard errors seem rather small, even if the number of patients and the homogeneity of the groups are considered. How do the authors explain these small standard errors?
Response: We do strongly believe that SE values of measured parameters are not small since they range within 10 to 30% of mean value.

• The patients with TB infection did not show increased expression of TREM-1, and this finding is not consistent with a report in which BCG increases TREM-1expression (Infection and Immunity 2004;72:937). Could the authors briefly mention the clinical characteristics of these patients?
Response: The manuscript now reads, on pg9, ln3-4: “Patients suffering from tuberculosis and enrolled in group B were presented with pleuritis.”

• The results shown in Table 1 represent the values in the first blood sample? If this is the case, where are the results from the second and third blood samples?
Response: These results have been omitted from Table 1. Table 2 was revised to include data of consecutive days of sampling.

• How many days did the non-surviving patients stayed in the ICU?
Response: The manuscript now reads, on pg10, ln15-18: “Among patients admitted in the ICU, two died on the second day post-admission; one died on the third post-admission; one on the seventh day post-admission; one the eighth day post-admission; and one on the twentieth day post admission.”
• It is possible that the increased expression of TREM-1 could be related with disease severity (as assessed by increased SOFA and APACHE scores), and not with the presence of an infectious process?

**Response:** The manuscript now reads, on pg8, ln16-17: “Correlations between severity scores and measured parameters were done according to Spearman”. The manuscript also reads, on pg11, ln13-17: “Positive correlations were found between APACHE II scores and expression of TREM-1 on monocytes on day 1 ($r_s$: +0.363, p: 0.010); and between APACHE II scores and sTREM-1 on day 1 ($r_s$: +0.262, p: 0.043). No significant correlations were found between APACHE II scores and expression of TREM-1 on neutrophils on day 1 as well as between SOFA scores and any of the measured parameters on day 1.”

• It could be helpful if the authors comment how they performed the stains for flow cytometry.

**Response:** The manuscript now reads, on pg7, ln7-13: “Briefly, red blood cells were lysed by ammonium chloride. White blood cells were labelled by phycoerythrin-conjugated anti-TREM-1 monoclonal antibodies (R&D InC, Minneapolis, USA) for 30 minutes in the dark. nTREM-1 and mTREM-1 expression was assessed after passage of labelled cells through a flow cytometer (Epics XL/MSL, Beckman-Coulter Co, Miami Florida) and expressed as the mean fluorescence intensity (MFI) with gating for neutrophils and for monocytes by their characteristic FS/SS scattering.”

• The text refers to PCR results in Table 2, but in this table there are no PCR results. Do patients included in this table correspond to group A? What happened with nTREM-1 and mTREM-1 in the same group of patients?

**Response:** These data have been added in the fully re-written Table 2.
In figure 2, the differences in the sTREM-1 panel are with respect to the column of Gram(+)/Haemophilus? It could be helpful to show your results in graphs with box and whiskers.

Response: Panel of sTREM-1 of Figure 3 of the revised manuscript, i.e. Figure 2 of the original manuscript, was redrawn with values given as boxplots and the statistical comparisons clearly indicated.

The patients of group A had higher values of APACHE and SOFA than those in group B. Could it be possible that the increased Inflammatory mediators are related to the severity of the illness rather than to the presence of infection?

Response: The manuscript now reads, on pg8, ln16-17: “Correlations between severity scores and measured parameters were done according to Spearman”. The manuscript also reads, on pg11, ln13-17: “Positive correlations were found between APACHE II scores and expression of TREM-1 on monocytes on day 1 \(r_s: +0.363, p: 0.010\); and between APACHE II scores and sTREM-1 on day 1 \(r_s: +0.262, p: 0.043\). No significant correlations were found between APACHE II scores and expression of TREM-1 on neutrophils on day 1 as well as between SOFA scores and any of the measured parameters on day 1.”

In discussion: You mention that sTREM-1 levels were decreased in patients from group A after the initiation of appropriate antibiotic therapy, the improvement of clinical symptoms and the resolution of CAP. It would be interesting to know how much time does it take to see this reduction. In those patients that died, how do you explain the decrease of sTREM-1 in the second and third blood samples?

Response: The manuscript now reads, on pg12, ln7-13: “Mean ± SE of survivors of group A was 311.42 ± 107.67 pg/ml on day 1; it was decreased to 188.79 ± 80.26 pg/ml on day 3 (p: 0.005 compared with day 1); and it was decreased to 175.51 ± 79.22 pg/ml on day 7 (p: 0.005 compared with day 1). Mean ± SE of non-survivors of group A was 140.66 ±35.90 pg/ml on day 1; it
was unchanged on day 3 \((145.79 \pm 45.29 \text{ pg/ml}, p: \text{NS compared with day 1})\); and it was unchanged on day 7 \((123.71 \pm 62.77 \text{ pg/ml}, p: \text{NS compared with day 1})\)”. The revised manuscript also reads on pg13, ln6-8: “sTREM-1 levels were decreased within the first 48 hours in patients with CAP with favourable outcome probably after the initiation of appropriate therapy followed by improvement of clinical symptoms.”

- **There are a lot of issues with these results that are not sufficiently explained in discussion, and there are some results that should be shown, especially those for day 3 and 7 blood samples.**

**Response:** Table 2 was revised to include data of consecutive days of sampling.