Author's response to reviews

Title: Endothelin-1 precursor peptides correlate with severity of disease and outcome in patients with community acquired pneumonia

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Author's response to reviews: see over
Dear Annabel

We thank you for your helpful review of our manuscript and gratefully accept your proposition to publish our work in the BMC Infectious Diseases.

Please find attached the responses to the reviewer comments and an updated version of the manuscript with changes highlighted. We hope that the manuscript is now acceptable for publication in BMC Infectious Diseases.

With best regards,

Philipp
Response to Reviewer 1 (Prof. Vincent)

1. The authors are too enthusiastic about their findings – An AUC of only 0.64 for mortality does not yield a high predictive value. The clinical implications are quite limited. The tone should be turned down and the discussion section could be shorter.

Response: Indeed, at this stage, the clinical implications of proET-1 are limited and further intervention studies are needed to study its clinical usefulness. Accordingly, we stated these points as limitations and have rephrased the discussion section (page 11, 13, 14 and 15).

2. Introduction line 8: “shows beneficial effects “ this statement is too strong – this is not an established treatment of septic shock.

Response: We rephrased the sentence in the revised manuscript to: “In addition, preliminary data suggested a potential beneficial effect of ET-1 antagonism by using a selective ET receptor antagonist during septic shock [6-9]” (page 3).

3. Results: what were the corresponding values (AUC) for PCT?

Response: Procalcitonin had a prognostic accuracy (AUC) of 0.59 [95% 0.51-0.67] for death and 0.65 [95% 0.57-0.72] for adverse outcome including death and ICU admission. Since this manuscript focuses mainly on proET-1 and the clinical scores, we included these numbers only in the text (result section and abstract, page 1 and 9) but not in table 2.

4. Does renal failure influence proET-1 levels? This should be discussed;

Response: We thank the reviewer for this important comment. Previous studies (e.g. Weitzberg, 1991) have found increased levels of endothelin-1 in patients with renal failure. Renal insufficiency probably further increases proET-1 levels in patients with severe sepsis and volume depletion caused by vasodilation. As renal failure correlates with adverse outcome in patients with CAP according to the PSI and the CURB65 score, this mechanism might even improve the prognostic accuracy of proET-1. Accordingly, we rephrased the paragraph (page 12) to:” Endothelin has been discussed as one of the main pathophysiological mechanisms underlying renal vasoconstriction during endotoxaemia [24, 25]. Increased levels of ET-1 have been found during sepsis related renal insufficiency and infusion of ET-1 has been shown to reduce renal and splanchnic blood flow in healthy volunteers[2, 26]. In addition, receptor antagonism of ET1 has been suggested to improve renal perfusion and function during sepsis[27]. Likewise, in our clinical study we found a significant correlation of ET-1 precursor peptides with creatinine and urea. ProET-1 levels may mirror both, the inflammatory cytokine response correlated with the severity of pneumonia, as well as the presence of disease-relevant comorbidities, namely renal dysfunction.”

5. The discussion section should indicate the superior prognostic value of proET-1 over PCT in this study.

Response: We integrated a statement concerning PCT and proET-1 in the discussion (page 12):“Procalcitonin has been put forward as a useful marker for
predicting disease severity and outcome in patients with pneumonia. [22, 23] Notably, in our study procalcitonin levels correlated with CAP severity as assessed by the PSI and the CURB65 score, however, procalcitonin did not reach the prognostic accuracy of proET-1 or the clinical scores. Based on our results, procalcitonin is rather a diagnostic tool able to guide decisions on antibiotic therapy, whereas proET-1 is a superior prognostic marker and predicts severity of disease”.

6. p 12: combining variables may be valuable; the authors seem to allude to it in the next page (p 13); this should come before the discussion about ET-1 blocking agents.
   **Response:** We rearranged the paragraphs on page 12 /13 accordingly.

7. Results: is the information about smokers so important?
   **Response:** We omitted the information about smoking status from the baseline Table and the result section.

8. Results: numbers could be provided numerically
   **Response:** We now present all numbers in the result section numerically.
Response to Reviewer 2 (Prof. Marshall)

1. First prognostication per se is of little clinical value if it cannot be tied to a decision that might alter that prognosis. Expressed differently, clinicians are less interested in knowing who is likely to live and die than they are in knowing what they might do to alter that probability. Markers such as C-reactive protein have been evaluated primarily on the basis of their capacity to predict the presence of invasive infection prior to the availability of blood cultures, rather than to risk stratify patients. Secondly, even though there is evidence of prognostic capability, the strength of this predictive capacity is really quite modest based on likelihood ratios and ROC curves.

Response: Indeed, the utility of a biomarker is defined by the degree by which it improves clinical decision making and therefore changes outcome of individual patients. As a retrospective analysis, this study can not provide such information, but may be hypothesis-generating for future prospective studies.

As stated by the reviewer, the prognostic accuracy of proET-1 per se is modest. Interestingly, the combination of proET-1 and the CURB65 risk scores in a multivariate logistic model significantly improved the prognostic accuracy of the new model. We therefore believe that combining proET-1 with established clinical scores might be the most promising future utility of this biomarker.

We stated these limitations and rephrased the discussion section (page 14).

2. In the introduction, this focus appears to be on prognostication rather than diagnosis. As mentioned above, a reader will be more interested in what new information is being provided by this marker.

Response: Due to the study design we can not provide information whether Pro-ET1 is a good marker to diagnose pneumonia. To provide this information a proper control group without pneumonia but with e.g. chest X-rax infiltrate is required. However, in the revised manuscript we now provide data about the diagnostic accuracy of Pro-ET1 to predict bacteraemia. We added a paragraph in the result section (page 8) on the predictive value of proET-1 to diagnose bacteraemia in patients as compared to procalcitonin: “The diagnostic accuracy to predict bacteraemia of procalcitonin (AUC 0.84 [95% 0.74-0.93]) was in the range of ProET1 (AUC of 0.77 [95%CI 0.67-0.86], p=0.21) and superior than C-reactive protein (AUC 0.67 [95%CI 0.56-0.78], p=0.004) and leukocyte count (AUC0.66 [95%CI 0.55-0.78], p=0.03). At an optimal cut off of 154 pmol/L, proET-1 had a sensitivity and specificity to predict bacteraemia of 62% and 87% and a positive and negative predictive value of 37% and 95%. Likewise, procalcitonin had a sensitivity and specificity of 86% and 74% and a positive and negative predictive value of 27% and 98% at an optimal cut off of 1.34 µg/L.”.

In addition the diagnostic aspect of Pro-ET1 was mentioned in the Introduction (page 3).

3. The need for ICU admission is used as one of the outcome variables for the study. However there is no intrinsic need for ICU admission for critically ill patients; rather clinicians decide to admit patients to the ICU because their disease is sufficiently severe that they would benefit from technologies provided in the ICU such as mechanical ventilation or cardiovascular support. It would
therefore be of much more interest to know what specific dimension of illness is being captured in the need for ICU admission. Do proET-1 levels correlate with respiratory failure, with cardiovascular failure, or with some other discrete biologic aspect of organ dysfunction?

**Response:** This is a very interesting point raised by the reviewer. As all patients included in this study had CAP as their principal diagnosis, respiratory failure and thus need for non-invasive and invasive ventilation and hemodynamic problems were the predominate reasons for ICU admission. We added this information in the results section (page 8/9) as follows: 

"The reasons for ICU relocations were need for invasive (n=7) and non-invasive (n=17) ventilation and hemodynamic stabilization because of sepsis related hypotension (n=12). Ten patients who were admitted to the ICU subsequently died".

The decision to transfer a patient to the ICU, however, was up to the discretion of the treating physician and his superiors based on usual practice and without interference of the research team. Interestingly, although the true clinical need for ICU admission can be questioned, the capacity of ET-1 to predict ICU admission was rather good, based on the fact that the clinician was blinded.

4. In the introduction, you state that, “ET-1 is a key player in the pathogenesis of vascular dysfunction …” Please be more specific. Being “a player” is overly colloquial and uninformative. The writing style here and elsewhere in the paper is somewhat awkward, for example, beginning sentences with words such as “thereby”, and “accordingly”. These really aren’t causally related thoughts.

**Response:** We rephrased the introduction as follows (page 3): "Endothelin-1 (ET-1) is a potent vasoconstrictor agent, synthesized mainly by endothelial cells[1, 2]. In the experimental setting, endotoxaemia induces the expression of endothelin precursors (prepro-Endothelin) mRNA in the heart and the lung[2][3]. In humans, elevated plasma levels of mature ET-1 are found during systemic infections and increased plasma ET-1 levels correlate with mortality risk [3-5]. In addition, preliminary data demonstrated beneficial effects of ET-1 antagonism by using a selective ET receptor antagonist during septic shock [6-9]."

Redundant “thereby’s“ and other words were deleted where appropriate.

5. You indicate that your hypothesis is that circulating ET-1 levels might predict adverse outcome. Why are you evaluating prognosis rather than diagnosis?

**Response:** Based on the suggestion of the reviewer, we added a paragraph in the result section on the diagnostic accuracy of proET-1 to diagnose bacteraemia in patients as compared to procalcitonin (see Response Nr 2). The presented patient population, however, was homogenous in terms of their principal diagnosis (CAP) and all included patients had an infiltrate in their chest X-ray. In addition, over 90% of the patients were septic according to the clinical definition. We therefore believe that the posthoc analysis of these patient data is more in favor of the prognostic aspect.

6. While correlations alone do not prove causality, they can if properly analyzed provide interesting insights into a potential causal role that could be tested experimentally.

**Response:** We are not sure whether we correctly understand the question of the
reviewer. All correlations are only hypothesis generating and do not prove causality. Thus, the diagnostic and prognostic accuracy of Pro-ET1 will have to be proven in future intervention studies. This was mentioned as a limitation of our study on page 14.

7. As I understand it, your study was performed using samples from an interventional study that employed PCT to determine the length of antibiotic therapy in patients with the community-acquired pneumonia. Did your population include patients from both study arms? And might the intervention (the decision to stop antibiotics) have impacted on subsequent outcomes, even in the absence of a significant effect in the primary study? For example, are pro-endothelin-1 levels influenced by circulating antibiotics?
   **Response:** We did not find an influence of the randomization of the underlying study on the outcome of patients with regard to death and/or ICU admission. We added these findings in the manuscript as follows: “Treatment allocation according to the protocol of the original study did not have a significant impact on all-cause mortality (OR 0.85 [95%CI: 0.42-1.74], p=0.67) or admission to the ICU (OR 0.85 [95%CI: 0.48-1.49], p=0.571) and was thus not considered any further in this analysis.” (page 4).
   In addition we did not find an influence of the antibiotic treatment on proET-1 levels on admission.

8. What was the follow-up period? It is stated in your manuscript as a median, but what was the planned duration of follow-up and how many were followed for the full period?
   **Response:** A follow-up examination was planned after 6 weeks in all patients with CAP. Out of the 298 patients, two patients did not complete the follow-up examination. In these two patients we counted the last day of the hospitalization as the time point of follow up. We specified this in the methods section (page 5) “For outcome assessment, a follow up examination was planned 6 weeks after study inclusion. All patients were followed-up for a median duration of 42 days [IQR 35-53]. Two patients did not complete follow up and their last day of hospitalization was counted as the time point of follow up”.

9. Table No. 1 is overly detailed. I would suggest emphasizing key demographics and domains where proET1 shows differential performance characteristics or differential diagnostic or prognostic capacity.
   **Response:** We rearranged Table 1.

10. You show that proET1 levels are higher at hospital admission and higher in patients with adverse outcome. Although the medians differ here, the spread of the data is large and the overlap is considerable. These comments apply to both Figures 1 and 2.
   **Response:** Figure 1, 2, and 3 show a considerable overlap between groups demonstrating the limitation of a single biomarker. However, we believe that in addition to a thorough clinical evaluation, a biomarker may help delineating patients at greater risk for adverse outcome.
   Displaying the different figures demonstrating aggregated data (median and IQR)
as well as individual proET-1 values for each patient gives the reader a comprehensive and honest summary of the properties of proET-1. The same would also apply for PSI points (see figure 1).

11. You speak about the correlation between proET1 levels and bacteraemia. This is a potentially useful observation. What are the positive and negative predictive values and the likelihood ratio here?

Response: We thank the reviewer for this comment. We have included the sensitivities and specificities and the corresponding likelihood ratios for proET-1 as compared to procalcitonin in the results section and in the discussion as follows (page 8) “The diagnostic accuracy to predict bacteraemia of procalcitonin (AUC 0.84 [95% 0.74-0.93]) was in the range of ProET1 (AUC of 0.77 [95%CI 0.67-0.86], p=0.21) and superior than C-reactive protein (AUC 0.67 [95%CI 0.56-0.78], p=0.004) and leukocyte count (AUC0.66 [95%CI 0.55-0.78], p=0.03). At an optimal cut off of 154 pmol/L, proET-1 had a sensitivity and specificity to predict bacteraemia of 62% and 87% and a positive and negative predictive value of 37% and 95%. Likewise, procalcitonin had a sensitivity and specificity of 86% and 74% and a positive and negative predictive value of 27% and 98% at an optimal cut off of 1.34 µg/L” and “We found increased levels of proET-1 in our patients with CAP depending on disease severity and a decrease of proET-1 levels during recovery. In addition we found a significant relationship of proET-1 with other markers of inflammation and infection such as procalcitonin, C-reactive protein and leukocyte count, and highest levels of proET-1 were found in bacteraemic patients. ProET-1 levels had a higher diagnostic accuracy with a high negative predictive value as compared to traditional biomarkers of infection (C-reactive protein and leukocyte
count) and in the range of procalcitonin to exclude growth of bacteraemia in blood cultures. This widens the findings of a prior study suggesting that due to its high negative predictive value, procalcitonin might help limiting the number of blood culture collections in patients with CAP [21].”

12. How do you interpret your observation that the strongest correlation with proET1 is with levels of urea? Also, please note that correlations are better expressed as R2 values which provide a measure of the degree of variability explained by the variable.

Response: As suggested, we changed the correlation to R2 values and added a paragraph on the correlation of proET-1 and renal failure as follows (page 12) " Sepsis is frequently associated with organ deterioration, especially renal and cardiopulmonary insufficiency[19]. Endothelin has been discussed as one of the main pathophysiological mechanisms underlying renal vasoconstriction during endotoxaemia [24, 25]. Increased levels of ET-1 have been found during sepsis related renal insufficiency and infusion of ET-1 has been shown to reduce renal and splanchnic blood flow in healthy volunteers[2, 26]. In addition, receptor antagonism of ET1 has been suggested to improve renal perfusion and function during sepsis[27]. Likewise, in our clinical study we found a significant correlation of ET-1 precursor peptides with creatinine and urea. ProET-1 levels may mirror both, the inflammatory cytokine response correlated with the severity of pneumonia, as well as the presence of disease-relevant comorbidities, namely renal dysfunction.”

13. Once again, in Figure #3 there is considerable overlap and measures of central tendency are not that useful.

Response: Please see comment to question 10.

14. You cite an area under the curve for proET1 of 0.64. This is not particularly good and most authors would want a value of at least .8 for a reliable measure. Similarly a likelihood ratio of 1.5 or 0.5 is really quite modest. The values are, moreover, established in a retrospective fashion rather than from a validation set, and would be predicted to be even lower in such a validation set.

Response: We agree with the reviewer on that important point. As mentioned in question Nr. 4, we believe that proET-1 levels should only be considered in conjunction with clinical data. We stated this more precisely in the discussion section. We mentioned this point as a limitation in the discussion (page 15).

15. You performed a multi-variate logistic regression analysis however there really weren’t enough events (death or ICU admissions) to undertake such an analysis, given that the conventional rule of thumb that there should be 10 events per predictor variables in a logistic regions equation.

Response: We agree with the reviewer’s comment and recalculated the multivariate logistic regression models separately for the PSI and the CURB65 score including now only 4 variables (for 41 patient endpoints). The results of the separate analysis remained similar (Table 3).

16. In the discussion on page #11 you state, “our clinical study results confirm
these experimental data …” Observed correlations do not apply confirmation of experimental data.  

**Response:** We rephrased this paragraph to “Likewise, in our clinical study we found a significant correlation of ET-1 precursor peptides with creatinine and urea. ProET-1 levels may mirror both, the inflammatory cytokine response correlated with the severity of pneumonia, as well as the presence of disease-relevant comorbidities, namely renal dysfunction.”. (page 13)

17. Later on the same page, you state, “as prognostic scores mainly aim to identify low risk patients suitable for hospital discharge ….” Prognostic scores are rarely if ever used to inform a clinical decision in an individual patient.  

**Response:** Indeed, in the ICU setting the APACHE scores were not designed for decision-making about individual patients. Heretics these days complain, that it is nevertheless inappropriately used for individual therapeutic decision, namely for Xigris.

The PSI score on the other hand “has primarily been developed to detect those patients who can safely be treated as outpatients” (ERS Guidelines, page 1147). Therefore, in this setting outside of ICU, CAP-severity scores support a clinician’s decision to hospitalize a patient or treat him as an outpatient.

As suggested by the reviewer, we rephrased this sentence to “As the PSI and the CURB65 score mainly aim to support physicians in identifying low risk patients suitable for hospital discharge”. 