Author’s response to reviews

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

Authors:

Philipp Schuetz (Schuetzp@uhbs.ch)
Daiana Stolz (StolzD@uhbs.ch)
Beat Mueller (happy.mueller@unibas.ch)
Nils Morgenthaler (N.Morgenthaler@Brahms.De)
Joachim Struck (j.struck@brahms.de)
Christian Mueller (muellerC@uhbs.ch)
Roland Bingisser (BingisserR@uhbs.ch)
Michael Tamm (TammM@uhbs.ch)
Mirjam Christ-Crain (Christmj@bluewin.ch)

Version: 3 Date: 28 August 2007

Author’s response to reviews: see over
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,
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-ray 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% CI 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
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 proET-1 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”.


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

Philipp Schuetz, Daiana Stolz; Beat Müller, Nils G. Morgenthaler, Joachim Struck, Christian Müller, Roland Bingisser, Michael Tamm and Mirjam Christ-Crain, MD

Department of Internal Medicine, University Hospital Basel, Switzerland

Clinic of Pneumology and Pulmonary Cell Research

Research Department, B.R.A.H.M.S AG, Biotechnology Center Hennigsdorf/Berlin, Germany

Funding research support: none

Corresponding author:

Dr. Philipp Schuetz
Dept. of Internal Medicine
University Hospitals
Petersgraben 4
CH-4031 Basel
Switzerland
Tel: +41 (0)61 265 5078
Fax: +41 (0)61 265 5100
Email: schuetzp@uhbs.ch

Running title: Endothelin-1 precursor peptides in community acquired pneumonia
**Background:** Circulating levels of endothelin-1 are increased in sepsis and correlate with severity of disease. A rapid and easy immunoassay has been developed to measure the more stable ET-1 precursor peptides proET-1. The objective of this study was to assess the diagnostic and prognostic value of proET-1 in a prospective cohort of mainly septic patients with community-acquired pneumonia.

**Methods:** 281 consecutive patients with community acquired pneumonia were evaluated. Serum proET-1 plasma levels were measured using a new sandwich immunoassay.

**Results:** ProET-1 levels exhibited a gradual increase depending on the clinical severity of pneumonia as assessed by the pneumonia severity index and the CURB65 scores (p<0.001 and p< 0.01). The diagnostic accuracy to predict bacteraemia of procalcitonin (AUC 0.84 [95% 0.74-0.93]) was superior than C-reactive protein (AUC 0.67 [95%CI 0.56-0.78]) and leukocyte count (AUC0.66 [95%CI 0.55-0.78]) and in the range of proET-1(AUC of 0.77 [95%CI 0.67-0.86]). ProET-1 levels on admission were increased in patients with adverse medical outcomes including death and need for ICU admission. ROC curve analysis to predict the risk for mortality showed a prognostic accuracy of proET-1 (AUC 0.64 [95%CI 0.53-0.74]), which was higher than C-reactive protein (AUC 0.51 [95%CI 0.41-0.61]) and leukocyte count (AUC 0.55 [95%CI 0.44-0.65]) and within the range of the clinical severity scores (AUC 0.69 [95%CI 0.61-0.76] and 0.67 [95%CI 0.57-0.77]) and procalcitonin (AUC 0.59 [95% 0.51-0.67] and 0.65 [95% 0.57-0.72]). ProET-1 determination improved significantly the prognostic accuracy of the CURB65 score (AUC of the combined model 0.69 [95%CI 0.59-0.79]). In a multivariate logistic regression model, only proET1 and the clinical severity scores were independent predictors for death and for the need for ICU admission.

**Conclusions:** In community-acquired pneumonia, ET-1 precursor peptides correlate
with disease severity and are independent predictors for mortality and ICU admission. If confirmed in future studies, proET-1 levels may become another helpful tool for risk stratification and management of patients with pneumonia.

**Trial registration:** ISRCTN04176397
Introduction

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 suggested beneficial effects of ET-1 antagonism by using a selective ET receptor antagonist during septic shock [6-9]. Regrettably, the analytical reliability of ET-1 measurements is cumbersome because it is instable at room temperature and its rapid clearance from the circulation limits its use in clinical routine. Recently, a new sandwich immunoassay has been introduced that measures the more stable precursor fragments proET-1 [10, 11]. Unlike the mature peptide, these precursors can be detected for hours in the circulation. Because of the stoichiometric generation, this "prohormone" correlates with the release of the active peptide [10], a condition similar to that of insulin and C-peptide. Thus, these precursor peptides can be used to indirectly measure the release of mature ET-1 in physiological and pathological conditions.

At present there are no clinical data available regarding the release of proET-1 during severe systemic infections other than sepsis. As community-acquired pneumonia (CAP) is the most important precursor of sepsis, we hypothesize that circulating proET-1 levels are increased during the acute illness and might predict adverse outcome in a well-defined cohort of 281 patients with CAP requiring hospitalization.
Material and Methods

Setting and Study population

The present study evaluated data and available plasma samples from 281 patients admitted to the emergency department with CAP from November 2003 through February 2005[12]. The primary endpoint of the study was antibiotic stewardship guided by procalcitonin as compared to standard recommended guidelines[12]. A predefined secondary endpoint was the assessment of prognostic factors and biomarkers in CAP. A detailed description of the study has been published elsewhere[12]. Briefly, patients admitted to the University Hospital Basel, Switzerland, a 950–bed tertiary care hospital with suspected CAP and age > 18 years were consecutively included in this study. Excluded were patients with cystic fibrosis, active pulmonary tuberculosis, hospital-acquired pneumonia and patients with severe immuno-suppression. Patients were examined on admission to the emergency department by a resident supervised by a board-certified specialist in Internal Medicine. Baseline assessment included clinical data and vital signs, assessment of patients' functional status using a visual analogue scale, comorbid conditions, and routine blood tests. In all patients, the Pneumonia Severity Index (PSI) and the CURB65 score were calculated[13, 14]. Forty-nine percent of the patients [N=138] were randomized to receive antibiotic treatment according to procalcitonin guidance and 51% patients [N=143] were allocated to the control group. Treatment allocation 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.

CAP was defined by the presence of recently acquired respiratory signs, core body temperature >38.0°C, auscultatory findings of abnormal breath sounds or rales, leukocyte count >10 or <4 x 10⁹ cells L⁻¹ and an infiltrate on chest radiograph[15].
Chest radiographs were screened by the physician in charge and reviewed by a senior radiologist, unaware of clinical and laboratory findings.

**Outcome**

For outcome assessment, a follow-up examination was planned 6 weeks after study inclusion. Patients were followed-up for a median duration of 42 days [IQR 35-53]. Two patients did not complete the follow-up and as the resolution of illness was uneventful during the hospitalisation, the day of discharge was counted as the time point of follow up. Patients who survived until follow-up were counted as survivors, whereas patients who died within the follow-up period were counted as non-survivors. Adverse medical outcome for this analysis was defined as death and need for Intensive Care Unit (ICU) admission from any cause.

**Microbial investigations**

The laboratory workup for the patients with CAP has been previously described [12]. Briefly, it included sputum samples for Gram stain and culture, two blood samples for culture and urine sample for detection of *Legionella pneumophila*.

**Measurements of proET-1 and other laboratory parameters**

Pro-ET1 was batch-measured in the plasma with a new sandwich immunoassay as described elsewhere (CT-proET1, BRAHMS AG, Hennigsdorf, Berlin, Germany) [10, 11]. The assay (normal reference range 44.3 ± 10.6 pmol/l) has an analytical detection limit of 0.4 pmol/l. C-reactive protein was measured in EDTA plasma on a Hitachi Instrument 917 (Roche Diagnostics, Rotkreuz, Switzerland). Procalcitonin was measured using 20 to 50 µL of plasma or serum by a time-resolved amplified cryptate emission (TRACE) technology assay (PCT Kryptor®, B.R.A.H.M.S. AG,
Hennigsdorf, Germany). The assay has a functional assay sensitivity of 0.06 µg/L, 3 to 10-fold above normal mean values.

**Statistical analysis**

Discrete variables are expressed as counts (percentage) and continuous variables as medians and interquartile Ranges (IQR) unless stated otherwise. Frequency comparison was done by chi-square test. Two-group comparison of normally distributed data was performed by Students t-test. For multigroup comparisons, one-way analysis of variance with least square difference for posthoc comparison was applied. For data not normally distributed, the Mann-Whitney-U test was used if only two groups were compared and the Kruskal-Wallis one-way analysis of variance was used if more than two groups were being compared. Receiver-operating-characteristics were calculated using STATA 9.2 (Stata Corp, College Station, Tex). Thereby, outcomes were either survival until follow-up or adverse medical outcome including death and need for ICU admission until follow-up, respectively. To estimate the potential clinical relevance of proET-1 measurements, we used likelihood-ratio tests to determine whether logistic regression models that included measurements of proET-1 and PSI/CURB65 provided a significant better fit than did logistic regression models limited to the PSI or CURB65 alone. Correlation analyses were performed by using Spearman rank correlation. Levels that were non-detectable were assigned a value equal to the lower limit of detection for the assay. All testing was two-tailed and P values less than 0.05 were considered to indicate statistical significance.
Results

Baseline parameters

The median age of the 281 patients was 74 years and 20 percent of patients were pretreated with antibiotics. Temperature ≥ 38°C was present in 64 percent of patients. Cough, increased sputum production and dyspnea, the typical self-reported cardinal symptoms of CAP, were present in 89, 74 and 75 percent of all patients, respectively. Overall, 80 percent of patients had relevant comorbidities including chronic obstructive pulmonary disease in 25 percent and coronary or hypertensive cardiopathy in 53 percent. The median PSI score at presentation to the Emergency Department was 100 [IQR 77-124] points. 39 percent of all patients were in the low risk PSI classes 1, 2 and 3. The median CURB65 score was 2 [IQR 2-3] and 47 percent of patients were in the low risk classes 0 and 1. 97 percent of patients were hospitalized for more than one night with a median length of hospital stay of 11 days [IQR 7-17]. Blood culture collection was performed in 89 percent of patients and in 12 percent growth of microorganisms was observed (S.pneumoniae (60%), S.aureus (10%), E.coli (6%), Klebsiella pneumoniae (6%)). Detailed baseline characteristics of the study population are summarized in Table 1.

ProET1 levels at presentation and recovery and correlation with disease severity

ProET1 (pmol/L) levels were significantly higher at hospital admission as compared to recovery (108.1 [IQR 99.3-117.0] vs 70.1 [IQR 64.6-71.5], p<0.0001 (Figure 1). On admission, pro-ET1 levels significantly increased with increasing severity of CAP as determined by the PSI scores and the CURB65 score (p<0.001 and p<0.01) (Figure 2). Median proET1 levels showed an about 2-fold increase from patients with PSI class 1 to PSI class 5, and a 0.4-fold increase from CURB65 class 0 to class 4, respectively. This gradual increase was also present for procalcitonin levels (p<0.01,
p<0.01) and total leukocyte count (p=0.03, p=0.004), but not for C-reactive protein (p=0.12, p=0.61) and body temperature (p = 0.59, p=0.42).

ProET-1 levels showed significant correlations with renal function (serum creatinine ($r^2=0.29, p<0.0001$) and urea levels ($r^2=0.32, p<0.0001$)) and with total length of hospital stay ($r^2=0.08, p<0.0001$) and inversely correlated with systolic blood pressure ($r^2=-0.049, p<0.01$).

Pro-ET1 levels as diagnostic markers of bacterial infection

ProET-1 (pmol/L) levels were significantly higher in patients with growth of bacteria in their blood culture (n=31) as compared to patients without growth of bacteria (160.0 [IQR 95.7-218.0] vs 92.4 [IQR 63.7-128.5], p<0.0001). ProET-1 levels significantly correlated with other biomarkers of infection, i.e. procalcitonin ($r^2=0.31, p<0.0001$), C-reactive protein ($r^2=0.11, p<0.0001$) and total leukocyte count ($r^2=0.07, p<0.001$).

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.

Pro-ET1 levels as prognostic markers for outcome

At follow-up, an adverse medical outcome was noted in 61 patients (22%), including 35 deaths (13%) and 36 admissions to the ICU (13%). The reason 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. In patients who died during follow-up, proET-1 (pmol/L) levels on admission were significantly higher as compared to levels in survivors (124.0 [IQR 91.7-199.0] vs. 92.9 [IQR 65.0-133.0], p=0.008) (Figure 3a). The respective values for other markers of infection were not significant (for procalcitonin: 0.61 [IQR 0.37-3.13] vs. 0.48 [IQR 0.18-2.45] µg/L (p=0.09), for C-reactive protein 152 [IQR 84-211] vs. 132 [IQR 66-213] mg/L (p=0.83) and for total leukocyte count (13.5 [IQR 10.3-16.9] vs. 12.7 [IQR 9.0-16.7] x10^9/L (p=0.35)). Moreover, proET1 levels but not procalcitonin, C-reactive protein and leukocyte count were elevated in patients with an adverse medical outcome consisting of either death or ICU admission (129.0 [IQR 94.6-191.0] vs. 88.2 [IQR 63.4-128.0] pmol/L, p<0.0001) (Figure 3b).

To assess the prognostic ability of proET-1 to predict (a) death and (b) adverse medical outcome including death and ICU admission, a receiver operating curve (ROC) was calculated. The discriminatory ability to predict death and adverse outcome of proET1 (AUC 0.64 [95%CI 0.53-0.74] and AUC 0.69 [95% 0.61-0.77]) was significantly better as compared to C-reactive protein (AUC 0.51 [95% 0.41-0.61] and AUC 0.58 [95% 0.51-0.66]) and leukocyte count (AUC 0.55 [95% 0.44-0.65] and AUC 0.57 [95% 0.49-0.65]) and tended to be better as compared to procalcitonin (AUC 0.59 [95% 0.51-0.67] and AUC 0.65 [95% 0.57-0.72]). As demonstrated in Table 2 the prognostic accuracy of proET-1 was in the range of both clinical assessment scores (PSI and CURB65). The optimal prognostic accuracy for pro-ET1 was at a cut off-of 94 pmol/L. With this cut-off, the sensitivity to correctly predict mortality until follow-up was 71 percent, the specificity 53 percent, the positive likelihood ratio (LHR+) 1.5 and the negative likelihood ratio (LHR-) 0.54. The respective values to predict adverse medical outcome were seventy-seven, fifty-six
percent and 1.8 and 0.4, respectively. To estimate the additive value of proET-1 on the two clinical scores to predict both outcomes, we calculated a multivariate logistic regression model combing the PSI and proET1, and the CURB65 and proET-1, respectively. As demonstrated in Table 3, proET-1 improved the CURB65 score for the adverse medical outcome (p=0.04) and tended to improve it for death (p=0.06). The combination of ProET-1 and the PSI score did not significantly improve the prognostic value of the PSI score alone (AUC 0.71 [95%CI 0.62-0.76], p=0.43 and AUC 0.75 [95%CI 0.68-0.81], p=0.39).

When entering proET-1, procalcitonin, C-reactive protein, and each one of the clinical severity scores in a multivariate logistic regression analysis, only proET-1 and a rise in one of the two risk scores were independent predictors of death and adverse medical outcome. Table 4 shows the respective odds ratios and significance levels of all variables.

Finally, to illustrate the capacity of proET-1 for risk assessment for patients admitted to the emergency room, we performed a comparison of survival and adverse medical outcome in patients with proET-1 below and above the optimal cut off value of 94 pmol/l by Kaplan-Meier survival curves. Patients with proET-1 levels above the optimal cut off had significantly lower survival rates and a higher risk for adverse medical outcome as compared to patients with levels below the cut off of 94 pmol/L (Figure 4).
Discussion

The main findings of this study are that circulating levels of ET-1 precursor peptides correlate with the severity of CAP, as assessed by the PSI and CURB65 scores, resolve during recovery of illness and predict the later finding of bacteraemia in patients with CAP. ProET-1 levels on admission are independent predictors of short term mortality and need for ICU admission with a moderate but superior prognostic accuracy as compared to commonly measured laboratory parameters. Importantly, proET-1 levels can improve the prognostic accuracy of the commonly used CURB65 score to predict adverse outcome.

ET-1 originates from a larger precursor peptide, which is first proteolytically processed to big ET-1 and further excised by the action of endothelin-converting enzyme[2]. In this study, the precursor fragment of ET-1 was assessed, because proET-1 fragments are stable at room temperature and can be detected for hours after the cleavage in the circulation, in contrast to mature ET-1, which is eliminated within minutes and therefore escapes detection in clinical routine[10, 11].

CAP accounts for almost 10% of the mortality and morbidity in hospitalized patients in western countries [16]. The majority of patients in our study fulfilled the clinical criteria for sepsis. Severe infections are characterized by an overwhelming immunoinflammatory response mediated by the release of different biologically active substances perpetuating an inflammatory cascade. Whilst different cytokines and toxins contribute to the extensive vasodilatation often seen in systemic infections, ET-1 as the most potent human vasoconstrictor counteracts these effects on the endothelial system with the purpose to assure blood pressure homeostasis and blood supply to the individual organs[1, 2]. However, accumulating evidence indicates that increased ET-1 levels as seen during sepsis rather contribute to the disturbance in blood pressure homeostasis causing multiorgan failure and eventually death [17, 18].
Increased levels of mature ET-1 have been found in different experimental and clinical models of sepsis[5, 19, 20]. 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].

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, but 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.

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.

We found that ET-1 precursor peptides predicted mortality in patients with CAP. This confirms previous findings reported in the setting of septic shock [4, 5, 28]. Interestingly, previous studies on endothelin found that rather the time course than a single time point measurement had a prognostic value for outcome in patients with sepsis [4, 5]. In our study, a single proET-1 measurement on admission was able to predict outcome in patients with CAP with a moderate predictive accuracy as measured by the c-statistic. It is however reasonable to believe that sequential ET-1 measurements would further improve the prognostic value of proET-1. In addition to predict mortality, this study found proET-1 to predict the need for ICU admission and correlated with the total length of hospital stay. As the PSI and the CURB65 score mainly aim to support physicians in identifying low risk patients suitable for hospital discharge, a biomarker that correlates with expanded adverse medical outcomes might be of particular interest for clinical decision making on admission.

In the assessment and management of CAP, knowledge of prognostic factors is crucial to estimate the risk for adverse medical outcome and, thus the need for hospitalization. The PSI and the CURB65 score are extensively validated and valuable tools for this purpose[13, 14]. However, calculation of the PSI is laborious and prone to individual errors. In addition, it is heavily influenced by age and co-morbidities which are static parameters not reflecting the actual disease state[13]. The CURB65 score on the other hand is much easier to calculate, but dichotomises continuous variables with the risk of oversimplification and, thus, has an inferior prognostic accuracy[14]. In this context, there is interest for new measurable
biomarkers mirroring distinct pathogenetic mechanisms to predict severity and outcome in CAP. The utility of a biomarker in this context is defined by the degree it improves clinical decision making and adds timely information beyond that of readily available information from clinical examination[29]. Our retrospective study can not provide such information, but may help to provide a rationale for future prospective studies. The information of a biomarker may provide new insights into the pathophysiology and prognosis of the disease process facilitating risk stratification and monitoring of therapy as a surrogate outcome measure. In the future, a biomarker might help in delineating distinct populations of patients with discrete pathologies – a prerequisite to enable the targeted application of specific biologically rational therapies, i.e. ET-1 blocking agents like bosentan (Tracleer®). [6, 8, 29, 30] Our study demonstrates that ET-1 precursor peptides potentially are of diagnostic and prognostic utility as early and independent risk predictor for death and need for ICU admission in patients with CAP. The combination of proET-1 and the CURB65 risk scores in a multivariate logistic model significantly improved the prognostic accuracy of the new model.

It is advisable to base the difficult task of prognostic assessment and treatment decisions on several and not only one parameter, each mirroring different pathophysiological aspects. The findings of this study support and extend other observational studies evaluation the benefit of novel biomarkers (e.g. natriuretic peptides, adrenomedullin and vasopressin precursor peptides) in the diagnostic assessment and risk stratification of patients with cardiovascular and infectious disease[31-37]. However, intervention studies are needed to evaluate if the gain of information translates to an improved management of patients with CAP and if distinct patients may particularly benefit from proET-1 measurement. Importantly, antagonism of the endothelium system by bosentan has shown
beneficial effects in experimental models of sepsis[7, 8, 38, 39]. If these antagonistic therapies find their ways into the clinic, it might be of particular interest to see if the measurement of ET-1 levels could identify those patients who possibly profit the most from such therapies.

Some limitations should be considered in interpreting our results. First, this is a preplanned post-hoc analysis of the ProCAP study [12] and, thus, rather hypothesis-generating than definite. Second, one should be aware that an AUC of 0.64 is rather low to be of immediate clinical applicability and the number of events in this analysis is of only moderate size limiting the statistical power. For both reasons, our results need to be confirmed in future prospective studies with medical outcome as the primary endpoint.

In conclusion, systemic ET-1 precursor peptides correlate with disease severity and short term outcome in patients with CAP. Intervention studies are needed to show whether proET-1 measurement improves risk prognostication and thus improves the clinical management of patients with CAP.
Competing interests

BM and DS have served as consultants and received payments from Brahms to attend meetings related to the trial and for travel expenses, speaking engagements, and research. NM and JS are employees of Brahms. All other co-authors declare that they have no competing interests.

Authors` contributions

MCC and BM had the idea for the study and directed study design, data collection and analysis and writing of the report. PS analyzed the data and wrote the report. NM and JS did the analyses and helped in analyzing and writing of the report. DS, RB, CM and MT had substantial contributions in planning of the study, data collection, interpretation of data and/or writing of the manuscript.

Acknowledgements

We thank the staff of the clinics of emergency medicine, internal medicine and endocrinology and the department of clinical chemistry, notably Fausta Chiaverio, Martina-Barbara Bingisser, Maya Kunz, Vreni Wyss and Ursula Schild, for most helpful support during the study.
References


32. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Muller B: *Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an
### Table 1 Baseline Characteristics of the 281 Patients with CAP

#### Demographic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – years – [median-IQR]</td>
<td>74 [61-82]</td>
</tr>
<tr>
<td>Male sex - no. [%]</td>
<td>175 [62]</td>
</tr>
</tbody>
</table>

#### Coexisting illnesses - no. [%]

- Heart disease                  | 149 [53]       |
- Renal dysfunction              | 76 [27]        |
- Chronic obstructive pulmonary disease | 71 [25]      |

#### Clinical examination

<table>
<thead>
<tr>
<th>Finding</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confusion - no. [%]</td>
<td>25 [9]</td>
</tr>
<tr>
<td>Systolic blood pressure mmHg – [median-IQR]</td>
<td>130 [112-142]</td>
</tr>
<tr>
<td>Heart rate [bpm] – [median-IQR]</td>
<td>96 [82-110]</td>
</tr>
<tr>
<td>Temperature [°C] – [median-IQR]</td>
<td>38.4 [37.7-39.2]</td>
</tr>
</tbody>
</table>

#### Laboratory findings – [median-IQR]

<table>
<thead>
<tr>
<th>Finding</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein [mg/L]</td>
<td>132 [67-212]</td>
</tr>
<tr>
<td>Procalciton [µg/L]</td>
<td>0.52 [0.20-2.45]</td>
</tr>
<tr>
<td>Leukocyte count [x10⁹]</td>
<td>12.8 [9.1-16.8]</td>
</tr>
<tr>
<td>ProET-1 [nmol/L]</td>
<td>94.6 [65.5-139.0]</td>
</tr>
</tbody>
</table>

#### Risk assessment

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSI points – [median-IQR]</td>
<td>100 [77-124]</td>
</tr>
<tr>
<td>PSI class – [median-IQR]</td>
<td>4 (3-4)</td>
</tr>
<tr>
<td>CURB65 – [median-IQR]</td>
<td>2 (1-2)</td>
</tr>
</tbody>
</table>

IQR denotes Interquartile Range, PSI Pneumonia Severity Index
Table 2a: Prediction of death \((n=35)\) in patients with CAP \((n=281)\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>0.64</td>
<td>0.53-0.74</td>
<td></td>
</tr>
<tr>
<td>PSI score</td>
<td>0.69</td>
<td>0.61-0.76</td>
<td>0.32</td>
</tr>
<tr>
<td>PSI /proET-1 (combined)</td>
<td>0.71</td>
<td>0.62-0.76</td>
<td>0.04</td>
</tr>
<tr>
<td>CURB65 score</td>
<td>0.67</td>
<td>0.57-0.77</td>
<td>0.51</td>
</tr>
<tr>
<td>CURB65/proET-1 (combined)</td>
<td>0.69</td>
<td>0.59-0.79</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 2b: Prediction of adverse outcome \((n=61)\) in patients with CAP \((n=281)\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>0.69</td>
<td>0.61-0.77</td>
<td></td>
</tr>
<tr>
<td>PSI classes</td>
<td>0.71</td>
<td>0.65-0.78</td>
<td>0.57</td>
</tr>
<tr>
<td>PSI /proET-1 (combined)</td>
<td>0.75</td>
<td>0.68-0.81</td>
<td>0.06</td>
</tr>
<tr>
<td>CURB65 score</td>
<td>0.66</td>
<td>0.58-0.73</td>
<td>0.45</td>
</tr>
<tr>
<td>CURB65/proET-1 (combined)</td>
<td>0.69</td>
<td>0.62-0.77</td>
<td>0.75</td>
</tr>
</tbody>
</table>

CAP Community-acquired Pneumonia, AUC, Area Under the Curve; CI, Confidence Interval, PSI Pneumonia Severity Index, CURB65 Confusion – Urea – Respiration rate - Blood pressure - Age 65
### Table 3a: Prediction of mortality in multivariate regression analysis including the PSI score in patients with CAP (n=281)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>1.006 (1.0010-1.0011)</td>
<td>0.018</td>
</tr>
<tr>
<td>PSI</td>
<td>2.113 (1.308-1.412)</td>
<td>0.002</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>0.962 (0.901-1.026)</td>
<td>0.246</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.998 (0.993-1.001)</td>
<td>0.274</td>
</tr>
</tbody>
</table>

### Table 3b: Prediction of mortality in multivariate regression analysis including the CURB65 score in patients with CAP (n=281)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>1.007 (1.001-1.011)</td>
<td>0.010</td>
</tr>
<tr>
<td>CURB65</td>
<td>1.859 (1.214-1.847)</td>
<td>0.004</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>0.967 (0.910-1.026)</td>
<td>0.265</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.998 (0.993-1.001)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

### Table 3c: Prediction of adverse medical outcome in multivariate regression analysis including the PSI score in patients with CAP (n=281)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>1.007 (1.002-1.001)</td>
<td>0.004</td>
</tr>
<tr>
<td>PSI</td>
<td>2.092 (1.434-3.051)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>0.976 (0.950-1.004)</td>
<td>0.093</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.001 (0.998-1.004)</td>
<td>0.428</td>
</tr>
</tbody>
</table>

### Table 3d: Prediction of adverse medical outcome in multivariate regression analysis including the CURB65 score in patients with CAP (n=281)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProET-1</td>
<td>1.007 (1.003-1.012)</td>
<td>0.001</td>
</tr>
<tr>
<td>CURB65</td>
<td>1.536 (1.096-2.153)</td>
<td>0.013</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>0.978 (0.952-1.003)</td>
<td>0.093</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.001 (0.998-1.004)</td>
<td>0.443</td>
</tr>
</tbody>
</table>

*CAP Community-acquired Pneumonia, PCT CI, Confidence Interval, PSI Pneumonia Severity Index, CURB65 Confusion – Urea – Respiration rate - Blood pressure - Age 65*
Figures

Figure 1
ProET-1 levels on admission and during/after recovery after 42 days [IQR 35-53]. Data are shown as box plots.

Figure 2
ProET-1 levels increase according to disease severity as represented by the PSI and the CURB65 scores. PSI denotes Pneumonia Severity Index.

Figure 3
ProET-1 levels in survivors and nonsurvivors and in patients with and without an adverse medical outcome including death and/or need for ICU admission.

Figure 4
Kaplan Meier Survival curves showing (a) the incidence of death and (b) the incidence of adverse medical outcome including death and/or ICU admission in patients with ProET-1 levels above and below 94 pmol/L. P=log rank test.