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Repeated measurements of endothelin-1 precursor peptides predict the outcome in community-acquired pneumonia

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Abstract *Purpose:* Excessive activation of the endothelium is associated with adverse outcomes in patients with systemic infections. Endothelium-associated peptides, such as endothelin-1 (ET-1), correlate closely with endothelial activation, and therefore serve as surrogate biomarkers. Our aim was to investigate precursor peptides of endothelin-1 (proET1) on admission and during follow-up on days 3, 5 and 7 in a prospective cohort of 925 patients with community-acquired pneumonia. *Methods:* We investigated the association of initial and follow-up proET1 and other prohormone levels with 30-day mortality and ICU admission in proportional Cox regression models with time-varying covariates adjusted for the pneumonia-severity-index (PSI), and calculated reclassification statistics. *Results:* The mortality rate and ICU admission rate were 5.4% (95% CI 3.9–6.8%) and 9.0% (95% CI 7.1–10.8%). ProET1 levels on admission and changes from baseline to day 3 were significant mortality predictors with adjusted hazard ratios of 10.5 (95% CI 2.9–38.6) and 28.4 (95% CI 7.0–115.1). Initial proET1 levels improved the PSI in reclassification statistics (net reclassification improvement of 0.29, $p < 0.0001$) and in c-statistics (from 0.79 to 0.83, $p < 0.01$). Changes of proET1 on day 3 improved the c-statistic of the combined model of PSI and initial proET1 from 0.80 to 0.85 ($p < 0.01$) and reclassification tables demonstrated a significant improvement (net reclassification improvement 0.44, $p < 0.0001$). Similar significant results were found for the risk for ICU admission. *Conclusions:* In community-acquired pneumonia, ET-1 precursor peptides on admission and changes from baseline to day 3 were independent predictors for mortality and ICU admission, and significantly improved the PSI. If verified in intervention studies, monitoring of proET1 may be helpful for endothelium targeting therapies and for risk stratification complementary to other prohormones.

Keywords Endothelin-1 · proET1 · Community-acquired pneumonia · Risk assessment · Pneumonia Severity Index

Abbreviations

ADH	Antidiuretic hormone
ADM	Adrenomedullin
ANP	Atrial-natriuretic peptide
ATS	American Thoracic Society
AUC	Area under the receiver operating characteristic curve
CAP	Community-acquired pneumonia
CI	Confidence interval
ET1	Endothelin1

HR	Hazard ratio	NRI	Net reclassification improvement	ROC	Receiver operating characteristic curve
ICU	Intensive care unit				
IDI	Integrated discrimination improvement	PSI	Pneumonia Severity Index	TRACE	Time-resolved amplified cryptate emission
IQR	Interquartile ranges	PCT	Procalcitonin		

Introduction

Emerging evidence points to a critical role of the endothelium in patients with systemic infections [1–4]. Endothelial activation in response to infection is associated with changes in hemostatic balance, leukocyte trafficking, vascular permeability, inflammation, and microcirculatory flow [5]. Endothelial dysfunction has been implicated in the progressive subcutaneous and body-cavity edema typically developing in patients with progression of sepsis and is thought to contribute to the development of septic shock. Although endothelial activation evolved as an adaptive host response to infection, it can become excessive and systemically disseminated. This results in the collapse of the endothelium, and thereby contributes to morbidity and mortality associated with systemic infections and sepsis. Endothelium-associated peptides, such as endothelin-1 (ET-1), correlate closely with endothelial activation, and therefore may serve as surrogate biomarkers [2]. Increased levels of ET-1 correlate with mortality in animals and in patients with sepsis [2, 6–8]. Conversely, studies suggested a beneficial effect of antagonising ET-1 with a selective ET receptor antagonist during septic shock [9–14].

An important drawback of ET-1 is its instability at room temperature and its rapid clearance from circulation, which has limited its use for patient monitoring in clinical routine. Recently, a new sandwich immunoassay has been introduced that measures the more stable precursor fragments of ET1 called proET1 [15, 16]. Unlike the mature peptide, these precursors can be detected for hours in the circulation. Previous smaller studies found increased initial proET1 levels in patients presenting to the Emergency Department with sepsis [17], community-acquired pneumonia (CAP) [18–20] and exacerbation of chronic obstructive pulmonary disease [21] and an association with mortality. However, these data need validation in a large independent patient cohort. In addition, to the best of our knowledge, there are no comprehensive clinical studies investigating the use of repeated proET1 measurements for longitudinal monitoring of patients with systemic infections.

We have previously reported about the prognostic use of different prognostic markers on admission in a large patient cohort with respiratory tract infections [22], particularly pro-adrenomedullin (ProADM) [23], pro-atrial-natriuretic peptide (ProANP) [24], precursors of anti-diuretic-hormone (copeptin) [23] and procalcitonin (PCT) [25]. The aim of the present study was to expand upon these initial results and focus on proET1 to investigate whether repeated measurements improve risk assessment and monitoring of CAP patients.

Methods

Study design and setting

As a predefined ancillary project, we used clinical data and measured proET1 levels in all patients with CAP enrolled in the multicenter ProHOSP study [22]. The design of the study has been reported in detail elsewhere [26]. In brief, from October 2006 to March 2008, a total of 1359 consecutive patients with presumed lower respiratory tract infection from six different hospitals located in the northern part of Switzerland were included. A total of 925 patients had definite diagnosis of CAP, the remaining patients suffered from chronic obstructive pulmonary disease or acute bronchitis. The primary objective of the trial was to assess the safety of a procalcitonin algorithm for antibiotic stewardship. A predefined secondary endpoint was to investigate the prognostic performance of proET1 for prediction of 30 day mortality.

Selection of participants

Patients >18 years with suspected lower respiratory tract infection as principal diagnosis on admission were eligible. In accordance with guidelines, lower respiratory tract infection was defined by the presence of at least one respiratory symptom (cough, sputum production, dyspnea, tachypnea, pleuritic pain) plus at least one finding during auscultation (rales, crepitation), or one sign of infection (core body temperature >38.0°C, shivering, leukocyte count >10 or <4G/l cells) independent of antibiotic pre-treatment. CAP was defined as lower respiratory tract infection with a new infiltrate on chest X-ray [27, 28]. Patients were examined on admission to the emergency department by a resident supervised by a board-certified specialist in internal medicine. The standardized baseline assessment included medical history, clinical examination, lab tests and chest X-ray. For all

patients with CAP, the Pneumonia Severity Index (PSI) was calculated on admission as described elsewhere [29]. The study protocol was approved by all local ethical committees, and written informed consent was obtained from all participants.

Study endpoint

The primary endpoint for this analysis is all cause mortality within 30 days following study inclusion. The secondary endpoint was transfer to the intensive care unit (ICU) within 30 days. Within ProHOSP, we provided previously proposed criteria for ICU admission based on the 2001 American Thoracic Society (ATS) criteria [28]. In brief, ICU admission was recommended in patients with severe CAP, defined as the presence of either one of two major criteria (need for mechanical ventilation, septic shock), the presence of two of three minor criteria (systolic blood pressure <90 mmHg, multilobar disease, PaO_2/FIO_2 ratio <250) or more than two CURB points. For COPD patients, ICU criteria included severe acidosis or respiratory failure ($pO_2 < 6.7$ kPa, $pCO_2 > 9.3$ kPa, pH < 7.3), no response to initial treatment in the emergency department or worsening mental status (confusion, coma) despite adequate therapy. Outcome assessment was standardized and monitored by an independent Safety and Monitoring Board consisting of three specialists in pneumology, infectious diseases and intensive care medicine as part of the protocol [26]. Survival status and ICU admission was assessed during hospital stay and by structured phone interviews at day 30 by blinded medical students.

Measurements of proET1 and other blood markers

Plasma was collected on admission and on days 3, 5 and 7 in plastic tubes containing ethylenediaminetetraacetic acid (EDTA). They were placed on ice and were then centrifuged at $3{,}000 \times g$; then plasma was frozen at −70°C until assayed at the end of the study. ProET1 was batch-measured in the plasma by a blinded laboratory worker with a new sandwich immunoassay as described elsewhere (CT-proET1, BRAHMS AG, Hennigsdorf, Berlin, Germany) [15, 16]. The assay has a normal reference range of 44.3 pmol/l (Standard deviation ±10.6) and an analytical detection limit of 0.4 pmol/l. ProADM, pro-ANP and copeptin were batch-measured in plasma with sandwich immunoassay as described elsewhere [30–33]. The assays have analytical detection limits of 0.08 nmol/l, 4.3 and 0·4 pmol/l, respectively. PCT was measured with a high sensitive time-resolved amplified cryptate emission (TRACE) technology assay (PCT Kryptor®, BRAHMS. AG, Hennigsdorf, Germany). The assay has a detection limit of 0.02 µg/l and functional assay sensitivity of 0.06 µg/l.

We used imputations to deal with missing marker values as described previously [23]. The imputation dataset consisted of all patients and all covariates included in the calculation of the PSI, marker values on day 0, 3, 5, and 7, randomization arm, final diagnosis, total antibiotic exposure, length of hospital stay as well as death, ICU admission, complication, or disease recurrence within 30 days of inclusion. Outcomes were also included in the imputation to avoid bias. We then used average values over five imputed datasets for the final analysis.

Hypothesis and statistical analysis

Our hypothesis stated that initial proET1 levels and changes from baseline to days 3, 5 and 7 were associated with adverse outcome in patients in regard to the primary and secondary endpoint. To test this hypothesis we performed several statistical analyses [34]. First, to account for the timely changes in marker level and also for the changing population at risk over time, we calculated proportional Cox regression analyses with time-varying biomarker covariate and report hazard ratios (HR). Common logarithmic transformation (i.e. base 10) was performed to obtain normal distribution for skewed variables (proET1 levels). We adjusted the models for initial PSI as this score reflects the state-of-art risk assessment in CAP patients. Cox models were calculated with step-wise inclusion of baseline proET1 levels and changes between baseline and day 3, 5 and 7. Changes in proET1 levels were preferred to absolute proET1 values at the different time points because of better model performance as evidenced by Akaike's information criterion (AIC). The proportional hazards assumption of Cox regression was evaluated by graphical display and analysis of the scaled Schoenfeld residuals [35].

Next, we assessed discrimination of proET1 by calculating the area under the receiver operating characteristic (ROC) curves (AUC) at the different time-points. We tested whether initial proET1 improves the performance of the PSI by comparing ROC curves of the joint logistic regression of initial proET1 and the PSI to ROC curves limited to the PSI only. Then, the effects of adding initial and follow-up proET1 levels to initial PSI were further assessed using reclassification methods [36]. The analyses used continuous variable information with evaluation of the effects on risk category reclassification for survivors and non-survivors during the follow-up interval. This approach separately analyzed the reclassification of persons who died and those who did not die during follow-up. Reclassification to a higher risk group was considered upward movement in classification for non-survivors. On the other hand, reclassification downward was considered a failure for non-survivors and vice versa for survivors. Improvement in reclassification was estimated by taking the sum of differences in proportions

of individuals reclassified upward minus the proportion reclassified downward for non-survivors and the proportion of individuals moving downward minus the proportion moving upward for survivors. For estimating meaningful a priori mortality risk categories, we used probabilities for 30 day mortality from the original PSI cohorts (i.e. 0.1, 0.6, 0.9, 9.3, 27%) [29]. We calculated the net reclassification improvement (NRI) which assesses improvement in reclassification over risk categories; we also assessed integrated discrimination improvement (IDI), which can be viewed as a continuous version of the NRI without the recourse to a priori defined risk categories. Finally, we investigated the prognostic performance of proET1 with other prognostic markers by comparing the AUC of the markers on admission and on day 3 for prediction of both endpoints.

All statistical analyses were done with SAS version 6.12 software (SAS Institute, Cary, NC) and STATA 9.2 (Stata Corp, College Station, Tex). All testing was two-tailed and p values less than 0.05 were considered to indicate statistical significance.

Results

Patient population

This study included a total of 925 CAP patients (41% women) with a median age of 73 years. Patients had a high burden of comorbid diseases with chronic heart failure being present in 17%, chronic renal failure in 22%, chronic obstructive pulmonary disease in 30% and diabetes in 17.5% of patients. More than 50% of patients were classified into PSI high risk classes IV or V. Overall, 5.4% (95% CI 3.9–6.8%) of patients ($n = 50$) did not survive the 30 days following study inclusion; of those, 17 patients died within the first 3 days. A total of 9.0% (95% CI 7.1–10.8%) of patients ($n = 83$) were transferred to the ICU; of those, 23 received vasopressors treatment for septic shock, 24 were intubated and 48 had non-invasive ventilation. Baseline characteristics and follow-up information of the cohort are presented in Table 1.

Initial median proET1 levels were 112 pmol/l (IQR 81–169) and about threefold higher compared to values previously published for healthy subjects [15, 16]. Median proET1 levels were about twofold increased in the 52 not-surviving patients compared to survivors on admission (213 vs. 110 pmol/l) and on all days of follow up (Fig. 1). The same was true for patients transferred to the ICU. Patients with positive blood cultures ($n = 73$) had significantly higher median proET1 levels on admission (187 (IQR 115–251) vs. 110 (80–162), $p < 0.0001$).

Table 1 Baseline characteristics and outcomes of patients ($n = 925$)

Demographic characteristics	
Age (years)[a]	73 (58–82)
Sex (male), no. (%)	544 (59%)
Coexisting illnesses, no. (%)	
Chronic heart failure	159 (17%)
Renal failure	206 (22%)
Chronic obstructive pulmonary disease	282 (30%)
Diabetes	162 (17.5)
Clinical findings	
Confusion, no. (%)	74 (9%)
Respiratory rate (breaths/minute)[a]	20 (16–25)
Systolic blood pressure (mmHg)[a]	132 (119–148)
Heart rate (beats/minute)[a]	95 (82–108)
Body temperature (°C)[a]	38.1 (37.2–38.9)
Initial risk assessment	
PSI points[a]	92 (68–116)
PSI class, no. (%)	
I, II, III	452 (49%)
IV	349 (38%)
V	124 (13%)
Initial blood analysis	
White blood cells ($\times 10^9$/l)	12.1 (9.0–16.4)
C-reactive protein (mg/l)	155 (75–252)
Procalcitonin (µg/l)	0.5 (0.2–2.7)
ProET1 (pmol/l)	112 (81–169)
Follow-up	
Outpatient treatment, no. (%)	52 (6%)
Length of stay in hospitalized patients[a]	8 (5–13)
30 days outcomes, no. (%)	
Any adverse events	134 (14.5%)
All cause mortality	50 (5.4%)
ICU admission	83 (9.0%)
Disease-specific complications	31 (3.4%)

PSI pneumonia severity index, *ICU* intensive care unit, *IQR* interquartile range

[a] Expressed as median (interquartile range)

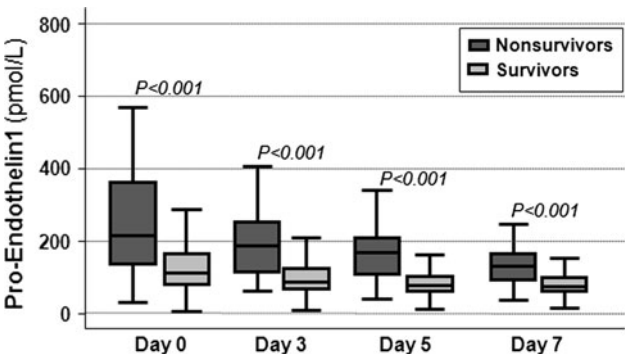

Fig. 1 Follow-up measurement of proET1 in non-survivors and survivors. The *lines* indicate median values, the *boxes* indicate upper and lower quartiles of the data, while the *whiskers* indicate the minimum and maximum values

Risk assessment with PSI and proET1 on admission

In a multivariable Cox regression model including PSI classes and initial proET1 levels (Table 2), both

Table 2 Multivariate Cox regression models for risk of death (left column) and ICU admission (right column) on days 1, 3, 5 and 7

	30-day mortality (HR, 95% CI)	*p* value	30-day ICU admission (HR, 95% CI)	*p* value
Multivariate Cox regression model on day 1				
PSI class	2.7 (95% CI 1.8–4.1)	**<0.0001**	1.1 (95% CI 0.9–1.4)	0.3587
ProET1 day 1	10.5 (95% CI 2.9–38.6)	**0.0004**	22.8 (95% CI 8.6–60.7)	**<0.0001**
Multivariate Cox regression model on day 3				
Pneumonia Severity Index	2.6 (95% CI 1.7–4)	**<0.0001**	1.1 (95% CI 0.9–1.4)	0.4102
ProET1 day 1	20.4 (95% CI 5.3–77.9)	**<0.0001**	25.3 (95% CI 9.5–67.4)	**<0.0001**
Change in ProET1 from day 1 to day 3	28.4 (95% CI 7–115.1)	**<0.0001**	21.7 (95% CI 2.9–164.9)	**0.0029**
Multivariate Cox regression model on day 5				
PSI class	2.6 (95% CI 1.7–4)	**<0.0001**	1.1 (95% CI 0.9–1.4)	0.3997
ProET1 day 1	20 (95%CI 5.2–76.6)	**<0.0001**	24.8 (95% CI 9.3–66.4)	**<0.0001**
Change in ProET1 from day 1 to day 3	45.5 (95% CI 6.2–335.5)	**0.0002**	46.6 (95% CI 6.2–349.6)	**0.0002**
Change in ProET1 from day 1 to day 5	0.5 (95% CI 0–5.7)	0.5814	0.1 (95% CI 0–143.1)	0.4908
Multivariate Cox regression model on day 7				
PSI class	2.6 (95% CI 1.7–4)	**<0.0001**	1.1 (95% CI 1–1.2)	**0.003**
ProET1 day 1	19.5 (95% CI 5.1–75)	**<0.0001**	14.9 (95% CI 6.4–34.7)	**<0.0001**
Change in ProET1 from day 1 to day 3	45.2 (95% CI 6.2–328.9)	**0.0002**	22.9 (95% CI 4.3–122.3)	**0.0002**
Change in ProET1 from day 1 to day 5	1.6 (95% CI 0.1–44.3)	0.7781	1 (95% CI 0–40.2)	0.9945
Change in ProET1 from day 1 to day 7	0.3 (95% CI 0.1–6.1)	0.4016	2.3 (95% CI 0–245.2)	0.7339

Note that hazard ratio (HR) corresponds to a unit increase in the explanatory variable; for proET1 this corresponds to an increase per unit of the log transformation of value; for the PSI this corresponds to an increase in PSI class
Bold values are significant ($p < 0.05$)

covariates were independent predictors for 30 days mortality with HRs of 2.7 (95% CI 1.8–4.1) per increase in PSI class and 10.5 (95% CI 2.9–38.6) per log unit increase of proET1. Initial proET1 significantly increased the AUC of the PSI classification (from 0.79 to 0.83, difference 0.04, $p < 0.01$). A reclassification table of the model with PSI class (old model) compared to a combined model with PSI class and initial proET1 levels (new model) confirmed a significant improvement in classification of patients (Table 3). A total of 16 non-survivors were classified into higher categories, while only one non-survivor was classified into a lower category. For survivors, 1 patient was classified into a lower category and 86 patients were classified into higher categories (see reclassification tables in the ESM). The respective NRI was 0.29 ($p < 0.0001$) and the IDI was 0.03 ($p < 0.0001$).

Risk assessment for mortality during follow-up

When stepwise adding the changes of proET1 between baseline (day 0) and days 3, 5 and 7 into the Cox regression model with baseline PSI class and baseline proET1 levels as time-dependent covariates, only the day 3 changes significantly improved the model, while day 5 and day 7 changes were not significant predictors (Table 2). ProET1 changes on day 3 also improved the AUC of the combined model of PSI class and initial proET1 levels from 0.80 to 0.847 (difference 0.049, $p < 0.01$) for discrimination of patients on day 3. In addition, a reclassification table confirmed that changes of proET1 levels on day significantly improved classification of patients compared to initial PSI and proET1 levels on day 3. The respective NRI was 0.44 ($p < 0.0001$) and the IDI was 0.037 ($p < 0.0001$).

ProET1 and risk for ICU admission

ProET1 on admission was significantly associated with ICU admission during follow up (HR 22.8 (95% CI 8.6–60.7), $p < 0.0001$, see Table 2**,** right column). While changes from baseline to day 3 were independent predictors in the Cox model, changes from baseline to days 5 and 7 did not further improve the model. ProET1 on admission and changes on day 3 were also found to significantly improve reclassification statistics of patients with and without ICU admission as demonstrated in Table 4.

We calculated sensitivity, specificity, positive and negative likelihood ratios at different cut offs of initial proET1 levels and changes in proET1 on day 3 for both endpoints (Table 5).

Prognostic performance of proET1 as compared to other prohormones

Finally, we compared the prognostic information of initial and day 3 proET1 levels with five prognostic biomarkers, namely the precursor levels of adrenomedullin (proADM), atrial-natriuretic peptide (proANP), antidiuretic hormone (copeptin), and procalcitonin. Table 6 shows results of ROC statistics for all markers as

Table 3 Characteristics of new and updated model on day 0 and day 3 for mortality prediction

	Old model	New model	New versus old model	*p*
Admission: comparison of PSI class alone with PSI class and initial proET1 level[a]				
Model Chi-square (log likelihoods)	62.2	74.6	12.4	**0.0004**
Area under the ROC curve	0.79	0.83	0.04	**<0.01**
Events				
Patients classified in higher categories			16	
Patients classified in same category			33	
Patients classified in lower categories			1	
No events				
Patients classified in higher categories			86	
Patients classified in same category			708	
Patients classified in lower categories			1	
Integrated sensitivity	0.12	0.15	0.03	**0.007**
Integrated specificity	0.05	0.048	−0.002	0.12
Integrated discrimination improvement			0.03 (SE 0.005)	**<0.0001**
Net reclassification improvement			0.29 (SE 0.08)	**<0.0001**
Day 3: comparison of PSI class and initial proET1 levels, with additional inclusion of proET1 changes[b]				
Model Chi-square (log likelihoods)	43	58.9	15.9	**<0.0001**
Area under the ROC curve	0.80	0.85	0.049	**<0.01**
Events				
Patients classified in higher categories			11	
Patients classified in same category			21	
Patients classified in lower categories			1	
No events				
Patients classified in higher categories			40	
Patients classified in same category			672	
Patients classified in lower categories			163	
Integrated Sensitivity	0.09	0.12	0.04	**0.002**
Integrated specificity	0.03	0.03	0.001	0.26
Integrated discrimination improvement			0.037 (SE 0.006)	**<0.0001**
Net reclassification improvement			0.44 (SE 0.10)	**<0.0001**

[a] *Old model* PSI class, *New model* PSI class and proET1 on day 1, *SE* standard error

[b] *Old model* PSI class and proET1 on day 1; *New model* PSI class, proET1 on day 1 and changes of proET1 on day 3; *SE* standard error

Bold values are significant ($p < 0.05$)

compared to proET1. Initial proET1 had a significantly higher AUC for mortality prediction compared to procalcitonin and a significantly higher AUC for ICU admission prediction compared to proANP and copeptin. The AUC of ProET1 on day 3 was superior compared to procalcitonin, but inferior to proANP and copeptin, while day 3 levels were better compared to proANP. ProADM showed a similar performance for ICU admission and for mortality prediction on admission, but day 3 values of proADM had a significnalty higher AUC for mortality prediction compared to proET1 ($p = 0.02$).

Discussion

In this study, we evaluated the prognostic information derived from repeated measurements of ET-1 precursor peptides for monitoring patients during the course of disease in a well defined cohort of CAP patients. Because a meaningful statistical assessment of such a research question is challenging due to the changing population at risk over time, we used a time-sensitive approach (Cox regression with time-varying covariates) and also calculated reclassification statistics for each time point. We found that both admission proET1 levels and relative changes between baseline and day 3 provided significant prognostic information in regard to mortality and the need for ICU admission and improved classification of the PSI score, while additional proET1 measurements on days 5 and 7 did not add any further prognostic information. The results also showed that relative changes in the marker level provided more information compared to absolute marker levels during the follow up.

Whilst different cytokines and toxins contribute to the extensive vasodilaton often seen in systemic infections, ET-1 as the most potent human vasoconstrictor counteracts these effects on the endothelial system with the purpose of assuring blood pressure homeostasis and blood supply to the individual organs [1–4]. However, accumulating evidence indicates that excessively increased ET-1 levels, as seen during sepsis, instead contribute to the disturbance in blood pressure homeostasis causing multiorgan failure and potentially death [1–4, 37, 38].

Table 4 Characteristics of new and updated model on day 0 and day 3 for prediction of ICU admission

	Old model	New model	New versus old model	*p*
Admission: comparison of PSI class alone with PSI class and initial proET1 level[a]				
Model Chi-square (log likelihoods)	22.5	62.9	40.4	**<0.0001**
Area under the ROC curve	0.65	0.74	0.09	**<0.01**
Events				
Patients classified in higher categories			19	
Patients classified in same category			50	
Patients classified in lower categories			14	
No events				
Patients classified in higher categories			75	
Patients classified in same category			548	
Patients classified in lower categories			219	
Integrated sensitivity	0.11	0.17	0.06	**<0.0001**
Integrated specificity	0.09	0.08	−0.005	0.02
Integrated discrimination improvement			0.06 (SE 0.008)	**<0.0001**
Net reclassification improvement			0.23 (SE 0.07)	**<0.0001**
Day 3: comparison of PSI class and initial proET1 levels, with additional inclusion of proET1 changes[b]				
Model Chi-square (log likelihoods)	12.4	21.6	9.3	**0.002**
Area under the ROC curve	0.77	0.82	0.06	**<0.01**
Events				
Patients classified in higher categories			2	
Patients classified in same category			8	
Patients classified in lower categories			0	
No events				
Patients classified in higher categories			68	
Patients classified in same category			508	
Patients classified in lower categories			254	
Integrated sensitivity	0.04	0.07	0.03	**0.04**
Integrated specificity	0.011	0.011	−0.0001	0.58
Integrated discrimination improvement			0.03 (SE 0.0006)	**<0.0001**
Net reclassification improvement			NA	

[a] *Old model* PSI class, *New model* PSI class and proET1 on day 1, *SE* standard error

[b] *Old model* PSI class and proET1 on day 1; *New model* PSI class, proET1 on day 1 and changes of proET1 on day 3; *SE* standard error

Bold values are significant ($p < 0.05$)

Table 5 Sensitivity, specificity, positive and negative likelihood ratio at different cut-offs

Cut point	Sensitivity (%)	Specificity (%)	Positive LR	Negative LR
Initial proET1 levels and risk for death				
100 (pmol/l)	82.00	39.54	1.36	0.46
150 (pmol/l)	68.00	71.09	2.35	0.45
200 (pmol/l)	52.00	85.60	3.61	0.56
Changes in proET1 levels (% from baseline) on day 3 and risk for death				
30% drop	78.79	33.49	1.18	0.63
No change	33.33	77.83	1.50	0.86
10% increase	24.24	88.11	2.04	0.86
Initial proET1 levels and risk for ICU admission				
100 (pmol/l)	85.54	40.74	1.44	0.35
150 (pmol/l)	61.45	71.97	2.19	0.54
200 (pmol/l)	45.78	86.46	3.38	0.63
Changes in proET1 levels (% from baseline) on day 3 and risk for ICU admission				
30% drop	80.00	31.93	1.18	0.63
No change	30.00	77.35	1.32	0.91
10% increase	30.00	88.19	2.54	0.79

Increased levels of mature ET-1 have been found in different experimental and clinical models of sepsis [8, 39, 40]. 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 [3]. In this study, the precursor fragment of ET-1

Table 6 Comparison of proET1 with other prognostic biomarkers

	AUC (95% CI)	*p**
Mortality prediction		
Day 1 marker levels		
ProET1 (pmol/l)	0.75 (95% CI 0.68–0.83)	
Procalcitonin (μg/l)	0.60 (95% CI 0.52–0.67)	**<0.001**
ProANP (pmol/l)	0.79 (95% CI 0.73–0.85)	0.32
Copeptin (pmol/l)	0.74 (95%CI 0.66-0.81)	0.61
ProADM (nmol/l)	0.76 (95% CI 0.68–0.83)	0.89
Day 3 marker levels		
ProET1 (pmol/l)	0.78 (95% CI 0.69–0.87)	
Procalcitonin (μg/l)	0.65 (95% CI 0.55–0.74)	**0.03**
ProANP (pmol/l)	0.76 (95% CI 0.69–0.84)	0.67
Copeptin (pmol/l)	0.86 (95% CI 0.8–0.92)	**0.03**
ProADM (nmol/l)	0.84 (95% CI 0.77–0.91)	**0.02**
ICU admission prediction		
Day 1 marker levels		
ProET1 (pmol/l)	0.74 (95% CI 0.68–0.79)	
Procalcitonin (μg/l)	0.69 (95% CI 0.64–0.75)	0.15
ProANP (pmol/l)	0.62 (95% CI 0.56–0.68)	**<0.001**
Copeptin (pmol/l)	0.69 (95% CI 0.63–0.75)	**<0.05**
ProADM (nmol/l)	0.72 (95% CI 0.65–0.78)	0.11
Day 3 marker levels		
ProET1 (pmol/l)	0.83 (95% CI 0.66–0.99)	
Procalcitonin (μg/l)	0.76 (95% CI 0.59–0.93)	0.49
ProANP (pmol/l)	0.73 (95% CI 0.59–0.86)	**<0.001**
Copeptin (pmol/l)	0.84 (95% CI 0.73–0.95)	0.86
ProADM (nmol/l)	0.82 (95% CI 0.65–0.99)	0.91

ProADM pro-adrenomedullin, *proANP* pro-atrial-natriuretic peptide, *copeptin* prohormone of anti-diuretic peptide
**p* value refers to comparison with proET1
Bold values are significant ($p < 0.05$)

was assessed, because these 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 [15, 16]. Previous CAP studies also assessed the prognostic performance of proET1 [17–19, 21]. A large study from Germany including 728 CAP patients reported a significant association of initial proET1 with 28 and 180 days mortality and respective AUC results of 0.79 and 0.76, which is within the range of our findings [19]. A previous smaller CAP study from our institution also found a correlation of proET1 with CAP severity and a fair prognostic accuracy for mortality prediction. The present study expands these previous findings to a multicenter CAP population and longitudinally repeated measurements. Based on the results of this and the previous studies, proET1 seems to be an interesting prognostic marker in patients with systemic infections, particularly CAP [17–19, 21].

CAP accounts for a significant burden of the mortality and morbidity in hospitalized patients in western countries, with >60,000 deaths in persons aged ≥15 years in the United States alone [41]. Timely and accurate prediction of outcome in individual patients with CAP based on clinical parameters is often challenging. Misclassification of patients may lead to both unnecessary hospitalisations of low-risk patients and adverse medical outcomes in high-risk patients. For this reason, guidelines recommend assessing the individual mortality risk by calculating severity scores [28], such as the PSI [42]. As evidenced within this and previous studies, the PSI has fair prognostic performance, which, however, leaves room for improvement [43]. There has been great interest in readily measurable prognostic blood biomarkers which could improve risk prediction [44]. We found that proET1 significantly improved the classification of patients with the PSI score on admission. In addition, the kinetic of proET1 from baseline to day 3 was also significantly associated with adverse outcome and improved risk classification of the initial PSI score and initial proET1 levels. This is in agreement with previous findings about repeated measurements of mature ET-1 reported in the setting of severest sepsis [7, 8, 45]. Interestingly, these investigators found that the time course, rather than a single time point measurement, had a prognostic value for outcome in patients with severe sepsis or septic shock [7, 8]. While initial assessment may help to guide decisions concerning inpatient or outpatient treatment, improved follow-up assessment of patients may help to identify candidates for safe early ICU or hospital discharge [46, 47].

Interestingly, antagonism of the endothelium system by bosentan has shown beneficial effects in experimental models of sepsis [9–14]. Studies found a significant beneficial effect of bosentan therapy in a septic shock mouse model in terms of survival, particularly during the later (hypodynamic) stages of septic shock [12]. Similarly, another study using a swine model found a correlation of cardiac depression with high endothelin levels, and a reversal upon bosentan treatment [13]. If these antagonistic therapies find their ways into clinical routine, it is tempting to hypothesize that measurement of proET1 levels may be able to identify those patients who might profit most from such therapies.

The combination of prognostic biomarkers and existing clinical scores has been suggested as a promising approach to increase the prognostic performance of severity scores in CAP [48]. Yet it still remains unclear which marker carries the most relevant information and is thus the best candidate marker for clinical decision making. When comparing proET1 to other prognostic prohormones including proADM, proANP, copeptin and procalcitonin, we found a significantly better performance of proET1 as compared to procalcitonin for mortality prediction, and only small differences compared to the other markers in question. We previously found that combining all markers together does not lead to large further improvements compared to including just a single marker [23]. Yet it is possible that proET1 may help to identify a specific high-risk patient population which would benefit from distinct therapies that specifically target the endothelium.

The strengths of this study are the thorough and predefined assessment of a large scale CAP patient cohort

from a multicenter study with repeated measurements of proET1 during the hospital stay. Still, there are residual limitations. Exclusion of some patients with dementia, immuno-suppression and active intravenous drug abuse and the study having been conducted only in Swiss hospitals limits generalizability to other settings and patients. This is of particular importance as other countries have a different spectrum of (resistant) bacteria for CAP which may influence the kinetics of proET1 over time. We used all-cause mortality in line with the original PSI cohort, and it is possible that proET1 would perform better, if only sepsis-related mortality were considered. We assessed endothelial dysfunction solely by means of plasma biomarkers and do not have other measures of direct vascular physiology. A previous study comparing endothelial biomarkers with skin biopsies suggested that caution is required in the interpretation of these markers in plasma, which does not necessarily reflect the in situ activation state of the endothelium [49]. Also, we did not assess pulmonary artery pressure, which may cause an increase in ET-1 levels independent from sepsis. Importantly, with an observational design, this study cannot address whether monitoring of proET1 would translate into better outcomes for patients. Therefore, prospective intervention studies need to be conducted to investigate whether proET1 measurements improve the daily clinical management of patients with CAP.

In conclusion, this study confirms the predictive value of proET1 on admission and on day 3 in conjunction with the PSI in regard to mortality in adult CAP patients. Future intervention studies must address whether adding proET1 to the PSI can increase its safe implementation in clinical practice and improve longitudinal patient monitoring.

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Appendix

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References

1. Lee WL, Slutsky AS (2010) Sepsis and endothelial permeability. N Engl J Med 363:689–691
2. Aird WC (2003) The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood 101:3765–3777
3. Shah R (2007) Endothelins in health and disease. Eur J Int Med 18:272–282
4. Wanecek M, Weitzberg E, Rudehill A, Oldner A (2000) The endothelin system in septic and endotoxin shock. Eur J Pharmacol 407:1–15
5. Aird WC (2008) Endothelium in health and disease. Pharmacol Rep 60:139–143
6. Hemsen A, Modin A, Weitzberg E (1996) Increased concentrations of endothelin-1 messenger RNA in tissues and endothelin-1 peptide in plasma in septic pigs: modulation by betamethasone. Crit Care Med 24:1530–1536
7. Brauner JS, Rohde LE, Clausell N (2000) Circulating endothelin-1 and tumor necrosis factor-alpha: early predictors of mortality in patients with septic shock. Intensive Care Med 26:305–313
8. Tschaikowsky K, Sagner S, Lehnert N, Kaul M, Ritter J (2000) Endothelin in septic patients: effects on cardiovascular and renal function and its relationship to proinflammatory cytokines. Crit Care Med 28:1854–1860

9. Wanecek M, Oldner A, Sundin P, Alving K, Weitzberg E, Rudehill A (1999) Effects on haemodynamics by selective endothelin ET(B) receptor and combined endothelin ET(A)/ET(B) receptor antagonism during endotoxin shock. Eur J Pharmacol 386:235–245
10. Krejci V, Hiltebrand LB, Erni D, Sigurdsson GH (2003) Endothelin receptor antagonist bosentan improves microcirculatory blood flow in splanchnic organs in septic shock. Crit Care Med 31:203–210
11. Oldner A, Wanecek M, Goiny M, Weitzberg E, Rudehill A, Alving K, Sollevi A (1998) The endothelin receptor antagonist bosentan restores gut oxygen delivery and reverses intestinal mucosal acidosis in porcine endotoxin shock. Gut 42:696–702
12. Iskit AB, Senel I, Sokmensuer C, Guc MO (2004) Endothelin receptor antagonist bosentan improves survival in a murine caecal ligation and puncture model of septic shock. Eur J Pharmacol 506:83–88
13. Wanecek M, Weitzberg E, Alving K, Rudehill A, Oldner A (2001) Effects of the endothelin receptor antagonist bosentan on cardiac performance during porcine endotoxin shock. Acta Anaesthesiol Scand 45:1262–1270
14. Weitzberg E, Hemsen A, Rudehill A, Modin A, Wanecek M, Lundberg JM (1996) Bosentan-improved cardiopulmonary vascular performance and increased plasma levels of endothelin-1 in porcine endotoxin shock. Br J Pharmacol 118:617–626
15. Struck J, Morgenthaler NG, Bergmann A (2005) Proteolytic processing pattern of the endothelin-1 precursor in vivo. Peptides 26:2482–2486
16. Papassotiriou J, Morgenthaler NG, Struck J, Alonso C, Bergmann A (2006) Immunoluminometric assay for measurement of the C-terminal endothelin-1 precursor fragment in human plasma. Clin Chem 52:1144–1151
17. Schuetz P, Christ-Crain M, Morgenthaler N, Struck J, Bergmann A, Müller B (2007) Circulating precursor levels of endothelin-1 and adrenomedullin, two endothelium-derived, counteracting substances, in sepsis. Endothelium 14:345–351
18. Schuetz P, Stolz D, Mueller B, Morgenthaler NG, Struck J, Mueller C, Bingisser R, Tamm M, Christ-Crain M (2008) Endothelin-1 precursor peptides correlate with severity of disease and outcome in patients with community acquired pneumonia. BMC Infect Dis 8:22
19. Kruger S, Ewig S, Giersdorf S, Hartmann O, Suttorp N, Welte T (2010) Cardiovascular and inflammatory biomarkers to predict short- and long-term survival in community-acquired pneumonia. Am J Respir Crit Care Med 182:1426–1434
20. Samransamruajkit R, Moonviriyakit K, Vanapongtipagorn P, Prapphal N, Deerojanawong J, Poovorawan Y (2002) Plasma endothelin-1 in infants and young children with acute bronchiolitis and viral pneumonia. Asian Pac J Allergy Immunol 20:229–234
21. Ando T, Ogawa K, Yamaki K, Hara M, Takagi K (1996) Plasma concentrations of atrial, brain, and C-type natriuretic peptides and endothelin-1 in patients with chronic respiratory diseases. Chest 110:462–468
22. Schuetz P, Christ-Crain M, Thomann R, Falconnier C, Wolbers M, Widmer I, Neidert S, Fricker T, Blum C, Schild U, Regez K, Schoenenberger R, Henzen C, Bregenzer T, Hoess C, Krause M, Bucher HC, Zimmerli W, Mueller B (2009) Effect of procalcitonin-based guidelines vs. standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. JAMA 302:1059–1066
23. Schuetz P, Wolbers M, Christ-Crain M, Thomann R, Falconnier C, Widmer I, Neidert S, Fricker T, Blum C, Schild U, Morgenthaler NG, Schoenenberger R, Henzen C, Bregenzer T, Hoess C, Krause M, Bucher HC, Zimmerli W, Mueller B (2010) Prohormones for prediction of adverse medical outcome in community-acquired pneumonia and lower respiratory tract infections. Crit Care (London, England) 14:R106
24. Vazquez M, Jockers K, Christ-Crain M, Zimmerli W, Muller B, Schuetz P (2010) MR-pro-atrial natriuretic peptide (MR-proANP) predicts short- and long-term outcomes in respiratory tract infections: a prospective validation study. Int J Cardiol [Epub ahead of print]
25. Schuetz P, Widmer I, Chaudri A, Christ-Crain M, Zimmerli W, Mueller B (2010) Prognostic value of procalcitonin in community-acquired pneumonia. Eur Respir J 37:384–392
26. Schuetz P, Christ-Crain M, Wolbers M, Schild U, Thomann R, Falconnier C, Widmer I, Neidert S, Blum CA, Schonenberger R, Henzen C, Bregenzer T, Hoess C, Krause M, Bucher HC, Zimmerli W, Muller B (2007) Procalcitonin guided antibiotic therapy and hospitalization in patients with lower respiratory tract infections: a prospective, multicenter, randomized controlled trial. BMC Health Serv Res 7:102
27. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM Jr, Musher DM, Niederman MS, Torres A, Whitney CG (2007) Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44(Suppl 2):S27–S72
28. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N, File T, Fine MJ, Gross PA, Martinez F, Marrie TJ, Plouffe JF, Ramirez J, Sarosi GA, Torres A, Wilson R, Yu VL (2001) Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 163:1730–1754
29. Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, Kapoor WN (1997) A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 336:243–250
30. Struck J, Morgenthaler NG, Bergmann A (2005) Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides 26:2500–2504
31. Morgenthaler NG, Struck J, Alonso C, Bergmann A (2005) Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem 51:1823–1829
32. Struck J, Tao C, Morgenthaler NG, Bergmann A (2004) Identification of an adrenomedullin precursor fragment in plasma of sepsis patients. Peptides 25:1369–1372
33. Morgenthaler NG, Struck J, Thomas B, Bergmann A (2004) Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. Clin Chem 50:234–236
34. Cook NR (2008) Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. Clin Chem 54:17–23
35. Grambsch PM, Therneau TM, Fleming TR (1995) Diagnostic plots to reveal functional form for covariates in multiplicative intensity models. Biometrics 51:1469–1482
36. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS (2008) Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 27:157–172 discussion 207–112
37. Grandel U, Grimminger F (2003) Endothelial responses to bacterial toxins in sepsis. Crit Rev Immunol 23:267–299

38. Kedzierski RM, Yanagisawa M (2001) Endothelin system: the double-edged sword in health and disease. Annu Rev Pharmacol Toxicol 41:851–876
39. Sanai L, Haynes WG, MacKenzie A, Grant IS, Webb DJ (1996) Endothelin production in sepsis and the adult respiratory distress syndrome. Intensive Care Med 22:52–56
40. Voerman HJ, Stehouwer CD, van Kamp GJ, Strack van Schijndel RJ, Groeneveld AB, Thijs LG (1992) Plasma endothelin levels are increased during septic shock. Crit Care Med 20:1097–1101
41. File TM Jr, Marrie TJ (2010) Burden of community-acquired pneumonia in North American adults. Postgrad Med 122:130–141
42. Fine MJ, Hough LJ, Medsger AR, Li YH, Ricci EM, Singer DE, Marrie TJ, Coley CM, Walsh MB, Karpf M, Lahive KC, Kapoor WN (1997) The hospital admission decision for patients with community-acquired pneumonia. Results from the pneumonia Patient Outcomes Research Team cohort study. Arch Intern Med 157:36–44
43. Schuetz P, Koller M, Christ-Crain M, Steyerberg E, Stolz D, Muller C, Bucher HC, Bingisser R, Tamm M, Muller B (2008) Predicting mortality with pneumonia severity scores: importance of model recalibration to local settings. Epidemiol Infect 136:1628–1637
44. Schuetz P, Christ-Crain M, Muller B (2009) Procalcitonin and other biomarkers to improve assessment and antibiotic stewardship in infections—hope for hype? Swiss Med Wkly 139:318–326
45. Albertini M, Clement MG, Hussain SN (2003) Role of endothelin ETA receptors in sepsis-induced mortality, vascular leakage, and tissue injury in rats. Eur J Pharmacol 474:129–135
46. Antonelli M, Azoulay E, Bonten M, Chastre J, Citerio G, Conti G, De Backer D, Lemaire F, Gerlach H, Hedenstierna G, Joannidis M, Macrae D, Mancebo J, Maggiore SM, Mebazaa A, Preiser JC, Pugin J, Wernerman J, Zhang H (2010) Year in review in Intensive Care Medicine 2009: I. Pneumonia and infections, sepsis, outcome, acute renal failure and acid base, nutrition and glycaemic control. Intensive Care Med 36:196–209
47. Andrews P, Azoulay E, Antonelli M, Brochard L, Brun-Buisson C, De Backer D, Dobb G, Fagon JY, Gerlach H, Groeneveld J, Macrae D, Mancebo J, Metnitz P, Nava S, Pugin J, Pinsky M, Radermacher P, Richard C (2007) Year in review in Intensive Care Medicine, 2006. II. Infections and sepsis, haemodynamics, elderly, invasive and noninvasive mechanical ventilation, weaning, ARDS. Intensive Care Med 33:214–229
48. Sligl WI, Majumdar SR, Marrie TJ (2009) Triaging severe pneumonia: what is the "score" on prediction rules? Crit Care Med 37:3166–3168
49. Leone M, Boutiere B, Camoin-Jau L, Albanese J, Horschowsky N, Mege JL, Martin C, Dignat-George F (2002) Systemic endothelial activation is greater in septic than in traumatic-hemorrhagic shock but does not correlate with endothelial activation in skin biopsies. Crit Care Med 30:808–814