

MR-proANP and Procalcitonin improve survival prediction in Ventilator Associated Pneumonia

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ABSTRACT

Ventilator associated pneumonia affects mortality, morbidity and cost of critical care. Reliable risk estimation might improve end of life decisions, resource allocation and outcome. Several scoring systems for survival prediction have been established and optimized over the last decades. Recently, new biomarkers gain interest in the prognostic field. We assessed whether MR-proANP and procalcitonin improve the predictive value of SAPS II and SOFA in ventilator associated pneumonia.

Specified endpoints of a prospective multinational trial including 101 patients with ventilator associated pneumonia were analyzed. Death within 28 days after ventilator associated pneumonia onset was the primary endpoint.

MR-proANP and procalcitonin on ventilator associated pneumonia onset were elevated in non-survivors as compared to survivors ($p=0.003$ and $p=0.017$, respectively) and their slope of decline differed significantly ($p=0.018$ and $p=0.039$, respectively). Patients with the highest MR-proANP quartile at ventilator associated pneumonia onset were at increased risk for death (log rank $p=0.013$). In a logistic regression model, MR-proANP was identified as the best predictor of survival. Adding MR-proANP and procalcitonin to SAPS II and SOFA improved their predictive properties (AUC 0.895 and 0.880).

We conclude that the combination of two biomarkers, MR-proANP and procalcitonin, improves survival prediction of clinical severity scores in ventilator associated pneumonia.

Key words: ventilator associated pneumonia, biomarker, MR-proANP, procalcitonin, risk stratification, prognosis

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INTRODUCTION

Ventilator associated pneumonia (VAP) remains an important cause of mortality despite therapeutical and preventive advances [1]. Up to one third of all mechanically ventilated patients develop pneumonia and mortality may exceed 50% in particular groups [2, 3]. Furthermore VAP is associated with a significant increase in hospital stay and excess costs outrange 40.000 dollars per patient [4]. Outcome in VAP is determined by several factors, including host characteristics, such as immunological status and comorbidities, intrinsic characteristics of the infection and therapeutical measures [5]. In this context, different clinical presentations, ranging from subtle to very obvious, combined to severe underlying diseases, make risk stratification in VAP particularly challenging. Several clinical severity scores such as APACHE, SAPS and SOFA have shown to predict outcome in critically ill patients [6]. However, most of them are time consuming and lack clinical usability. Recently, circulating biomarkers have been suggested for outcome prediction in infection [7, 8].

Pro Atrial Natriuretic Peptide (ProANP, 1-98), a stable fragment of the ANP precursor, is secreted in the same molar ratio as ANP [9]. ANP is a member of the natriuretic peptide family, which, together with the associated prohormones, comprises established markers of congestive heart failure [10]. NT-proANP has shown to be of prognostic relevance in cardiovascular disease and myocardial infarction [11]. Furthermore, a significant difference in proANP levels between survivors and non-survivors has been assessed in respiratory tract infections including pneumonia, sepsis and VAP [12-15].

Procalcitonin is a classical hormokine, which is secreted next to the hormonal pathway in a cytokine like manner [16]. Besides being well known for its diagnostic performance in bacterial conditions, procalcitonin also provides prognostic information [7, 17-19]. Accordingly, high serum procalcitonin and increasing procalcitonin levels were independent predictors of death in critical ill patients, including those with VAP [20-23].

Patients developing VAP are mechanically ventilated for severe underlying disease. Therefore, we investigated whether the combination of two biomarkers, assessing the hemodynamic (MR-proANP) and the infectious (procalcitonin) consequences of VAP, could add to the predictive value of clinical severity scores. We analyzed data of a well-characterized cohort of 101 patients with clinically diagnosed VAP. The primary end-point was death within 28 days after VAP-onset.

MATERIALS AND METHODS

Setting and study population

Data from a prospective multicentric trial including 101 patients with clinically diagnosed VAP were analyzed [24]. In brief, the main objective of the study was to evaluate procalcitonin-guided antibiotic de-escalation in VAP as compared to usual care. The study took place in seven medical and surgical ICUs (UMass Memorial Medical Center, Worcester; MA, USA; University Hospital Lausanne, Switzerland and University Hospital Basel, Switzerland). The analysis of prognostic predictors in the study population was a pre-defined secondary end-point of the protocol. The study was approved by the institutional review boards of all participating institutions and registered in the Current Controlled Trials Database as “ProVAP”-Study [ISRCTN61015974]. Written informed consent was obtained from all included patients or their legal representatives.

Diagnostic criteria

Diagnosis of VAP was established on a clinical approach, according to the American Thoracic Society Guidelines [1, 25]. It was defined as a new or progressive infiltrate on chest radiography associated with at least two of the following: purulent tracheal secretions; fever (body temperature $>38^{\circ}\text{C}$ / 100.4°F); leukocytosis/ -penia (leukocyte count $>11000/\mu\text{L}$ or $<3000/\mu\text{L}$). ICU patients were eligible for the study if they met all the following criteria: (1) intubated for mechanical ventilation for at least 48 hours; (2) older than 18 years; (3) clinical diagnosed VAP. Patients were excluded if they (1) were pregnant; (2) were enrolled in another trial; (3) had received immunosuppressants or long-term corticosteroid therapy (above 0.5 mg/kg per day for longer than 1 month); (4) were immunosuppressed; (5) had a coexisting

extrapulmonary infection diagnosed in the first three days and requiring antibiotic therapy for more than three days. Microbiologically confirmed VAP was defined by a significant growth of quantitative cultures of endotracheal aspirates (EA), bronchoalveolar lavage (BAL) or protected specimen brush (PSB) specimens [1].

Baseline Assessment and Follow-up

At time of enrolment the following information was recorded from each subject: age, gender, preexisting comorbidities, severity of the underlying medical condition(s), primary reason for initiating mechanical ventilation, duration of prior mechanical ventilation, antibiotic use within 14 days of VAP-onset, body temperature, heart rate, mean arterial pressure (MAP), oxygen saturation, ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen ($\text{PaO}_2/\text{FIO}_2$), leukocyte count (WBC), MR-proANP and procalcitonin serum levels. The following indices were calculated: simplified acute physiologic score II (SAPS II), sequential related organ failure assessment (SOFA) score, organ dysfunction and/or infection (ODIN) score, clinical pulmonary infection score (CPIS). During the 28 day follow-up period the following information was recorded: body temperature, heart rate, MAP, oxygen saturation, $\text{PaO}_2/\text{FIO}_2$, WBC, SOFA, ODIN and CPIS; mechanical ventilation status and antibiotic use and survival throughout the 28-day study period. Serum MR-proANP and procalcitonin levels were determined on VAP-onset and daily during 10 consecutive days after VAP diagnosis.

Outcome assessment

All patients were followed-up for 28 days or until death. Patients deceased before day 28 were classified as non-survivors, all others were classified as survivors. No patient was lost to follow-up.

MR-proANP and Procalcitonin measurements

MR-proANP measurements were performed with 50 μ L serum using a test based on the time-resolved amplified cryptate emission (TRACE) technology (MR-proANP KRYPTOR; BRAHMS AG; Hennigsdorf/ Berlin, Germany). The lower detection limit of the assay is 6 pmol/L and the functional assay sensitivity (defined as the lowest value with an interassay coefficient of variation less than 20%) is 23pmol/L [9]. The median MR-proANP level of healthy subjects was stated to be 45pmol/L. Procalcitonin was measured in 50 μ L serum using TRACE technology (PCT sensitive KRYPTOR; BRAHMS AG; Hennigsdorf/ Berlin, Germany). A lower detection limit of 0.02ng/mL and a functional assay sensitivity of 0.06ng/mL were identified [26].

Statistical Analyses

Discrete variables are expressed as counts (percentages) and continuous variables as median (interquartile range [IQR]). Comparability of groups was analyzed by Chi-Square Test, Fisher's Exact Test or Mann-Whitney U test, as appropriate. Correlation analyses were performed using spearman's rho. To detect the time-course of the biomarkers across survivors and non-survivors, a linear mixed-effect model with fixed factors day, group and random factor subject was performed on the log transformed parameters. In order to study possible different time-courses the interaction between day and group was determined. The (log-) values of the markers at VAP-onset were also included in the model to adjust for potential different baseline values in the study groups. Time to death was analyzed by Kaplan-Meier survival curves and compared by the Log rank test. Area under the curve (AUC) values for receiver operating characteristic curves were calculated from logistic regression models in which each

factor enters individually or combined. Values are calculated from a nonlinear regression model where each predictor is modelled as a five knot cubic spline [27]. AUC values were reported with corresponding standard errors (SE). To predict survival a L1-penalized logistic regression model was performed. This is an adaptation of Lasso regression to generalized linear models [28]. This approach is justified when the number of predictors is large compared to the number of observations and over-fitting has to be considered. Cut-offs with the highest accuracy were identified using the Youden's index (maximal difference between sensitivity and 1-specificity). Nomograms provide excellent graphical depictions of the variables in a regression model, in addition to enabling the user to obtain predicted values manually. A nomogram was calculated using R version 2.9.2. All tests were two tailed; $p < 0.05$ was defined as significant. Data were analyzed using statistical software (Statistical Package for Social Sciences, version 16 for Windows; SPSS, Chicago IL; R Development Core Team, version 2.9.2, Vienna).

RESULTS

Baseline Characteristics

A total of 101 patients were included in the study. Detailed baseline characteristics for survivors and non-survivors are summarized in **Table 1**. The median [IQR] age was 57 years [43-70]. Cardiac comorbidities were reported as follows: arterial hypertension (31), coronary artery disease (30), myocardial infarction (17), cardiac arrhythmia (16), congestive heart failure (11), aortic aneurysm (5). Microbiological analyses of respiratory tract secretions identified a causative organism in 74 patients (76%). The most frequently isolated bacteria were *Staphylococcus aureus* (30%), *Pseudomonas aeruginosa* (25%) and *Klebsiella* species (13%). 75% of all patients received antibiotics within 14 days prior to study inclusion. Appropriate initial antibiotic therapy, defined as a regimen combining an aminoglycoside or a fluoroquinolone plus a betalactam or an antipseudomonal carbapenem was applied in 86% of cases. Twenty patients died during the study period. Deaths were due to traumatic brain injury/subarachnoid hemorrhage (8), respiratory failure/ARDS (5), septic shock (3), cardiogenic shock (2), multiorgan failure (1) and acute liver failure (1).

MR-proANP in VAP

At VAP-onset median [IQR] MR-proANP was 163pmol/L [98-374]. Elevated MR-proANP was associated with age, heart rate, mean arterial pressure as well as with the presence of cardiac, pulmonary and renal comorbidities (**Table 2-3**). MR-proANP was significantly elevated in non-survivors (median [IQR]; 373pmol/L [114-784] versus 149pmol/L [93-278], $p=0.003$). There was a significant effect of the interaction between day and group, reflecting a different decrease of MR-proANP in

survivors and non survivors ($p=0.018$; **Figure 1A**). Kaplan-Meier survival function was significantly different across MR-proANP quartiles on VAP-onset (log rank $p=0.013$; **Figure 2**). In receiver operating characteristic (ROC) analysis, the area under the curve (AUC) of MR-proANP on VAP-onset to predict mortality was 0.801 (SE=0.062). A MR-proANP threshold of 660pmol/L had the highest accuracy for predicting death. The associated sensitivity and specificity were 45% and 97%, respectively. Accordingly, the likelihood ratio positive was 17.3. We assessed a positive and negative predictive value of 82% and 87%. The use of the 660pmol/L MR-proANP cut-off yielded an odds ratio of 30.7 (95% confidence interval: 5.9-161.0) for predicting death at VAP-onset.

Procalcitonin in VAP

Median [IQR] procalcitonin on VAP-onset was 0.69ng/mL [0.22-2.34] and significantly correlated with oxygen saturation, renal disease and gender (**Table 2-3**). Age, cardiac and pulmonary comorbidities did not influence procalcitonin levels. Procalcitonin levels were significantly elevated in non-survivors (1.36ng/mL [0.38-6.04] versus 0.58ng/ml [0.19-2.00], $p=0.017$). A significant effect of the interaction between day and group indicates a different decrease of the marker across the time ($p=0.039$; **Figure 1B**). The relative decrease (median) of procalcitonin within 72 hours after VAP-onset was 26% in survivors and 7% in non-survivors. Procalcitonin quartiles did not differ in survival (log rank $p=0.076$). The AUC for procalcitonin on VAP-onset to predict mortality was 0.712 (SE=0.069).

Combination of MR-proANP, procalcitonin and ICU-scores

At VAP-onset the AUC of SAPS II and SOFA to predict 28 day mortality were 0.736 SE=0.067 and 0.768 SE=0.065, respectively (**Figure 3A-B**). 25 parameters

such as age, vital signs, laboratory values, comorbidities, SAPS II, SOFA, ODIN, procalcitonin and MR-proANP serum levels on VAP-onset were included in a L1-penalized logistic regression model analyzing 28-day survival. MR-proANP was identified as the best predictor of survival, followed by SOFA and SAPS II. Adding MR-proANP on VAP-onset to clinical scores significantly improved the AUC for SAPS II to 0.859 (SE=0.055, p=0.024). The AUC of SOFA plus MR-proANP was enhanced to 0.848 (SE=0.056, p=0.095). A further refinement of the model was achieved by including procalcitonin on VAP-onset. SAPS II in conjunction with MR-proANP and procalcitonin significantly improved survival prediction as compared to SAPS II alone (AUC 0.895, SE=0.048, p=0.003; **Figure 3A**). Similarly, predictive properties of SOFA increased by adding MR-proANP and procalcitonin (AUC 0.880, SE=0.051, p=0.087; **Figure 3B**). In order to refine risk estimation in a clinically-feasible fashion we designed a two-dimensional diagram for risk stratification in VAP (**Figure 4**). In this nomogram, MR-proANP and SAPS II, assessed at VAP-onset, predict the risk of death within the following 28 days. Procalcitonin did not provide useful additional information.

The predictive values of MR-proANP, procalcitonin and clinical scores were also analyzed in the subgroup of patients with microbiologically confirmed VAP (n=74). As compared to patients with clinically diagnosed VAP, the AUC for procalcitonin on VAP-onset to predict mortality within 28 days was higher in patients with microbiologically diagnosed VAP (AUC 0.767 versus 0.712, SE=0.065). In contrast, the predicting property of MR-proANP at VAP-onset was similar in both populations (AUC 0.820 versus 0.801, SE=0.060). Combining MR-proANP with SOFA or SAPS II enhanced AUC values as compared to the single score (SOFA AUC 0.848, SE=0.056, p=0.071; SAPS II AUC 0.906, SE=0.046, p=0.019). The

conjunction of MR-proANP, procalcitonin and SOFA at VAP-onset predicted survival with a significantly better accuracy as compared to the SOFA score (AUC 0.900, SE=0.047, p=0.032).

DISCUSSION

We report three main findings: First, circulating MR-proANP and procalcitonin levels on VAP-onset are significantly elevated in non-survivors as compared to survivors. Second, a single MR-proANP determination on VAP-onset is a suitable marker for survival prediction. And finally, the combination of MR-proANP and procalcitonin improves survival prediction of SAPS II and SOFA, two well established ICU scores.

Circulating natriuretic peptides, such as ANP and BNP, are primarily of cardiac origin. They were mainly established for diagnostic purposes in congestive heart failure but have also been evaluated for prediction and treatment guidance [29-31]. Recently their diagnostic use in ICU is challenged [32]. However, natriuretic peptides gained relevance beyond cardiac disease. In intensive care natriuretic peptides were repeatedly suggested to predict survival [15, 33-35].

Elevated ANP levels have been reported in lower respiratory tract infections, sepsis and other pulmonary diseases [12-14, 36-38]. In VAP, we found elevated MR-proANP to be associated with cardiac, renal and pulmonary comorbidities. Congestive heart failure results in increased left cardiac pressures and volume overload. Similarly, acute pulmonary disease leads to transient pulmonary hypertension resulting in right heart strain [39]. Both represent main stimuli for ANP release. Renal failure elevates proANP levels most likely due to an inappropriate renal clearance [40]. Therefore, the associated underlying comorbidities in VAP lead to increased MR-proANP levels and represent an additional risk for death. VAP itself represents another ANP releasing stimulus. Accordingly, it was suggested that the

lung may possess its own “ANP system”, rather than being dependent on the circulating ANP [41]. Moreover, lymphoid organs have been suggested to secrete ANP [42, 43]. Recently, elevated TNF- α was associated with increased ANP independently of left ventricular function [44]. This indicates that TNF- α might exert direct cardiotoxic effects or that TNF- α may stimulate ANP release. Therefore, it is tempting to hypothesize that in inflammatory conditions, such as VAP, ANP secretion is partially mediated through cytokine release.

We have shown that MR-proANP is elevated in non-survivors and that the slope of decline differs significantly between survivors and non-survivors in VAP. Our results are in line with the findings reported by Seligman et al., suggesting MR-proANP to be associated with mortality and the severity of sepsis in a VAP cohort [15]. Noteworthy, the previous study has restricted the analysis to VAP patients presenting a clinical pulmonary infection score > 6 on day 3 and analyzed proANP levels only at two time points (day 0 and 4). In our cohort, MR-proANP levels were persistently increased over 10 days following VAP-onset in non-survivors. We report a similar mortality prediction of proANP in VAP as compared to Berdal et al., who assessed 70 unselected mechanically ventilated critically ill patients [35]. Thus, MR-proANP seems to reflect multiple comorbidities and infectious conditions related to survival in intensive care. Despite the exceptionally high mortality and resource utilization in VAP, specific tools assessing mortality are scarce [45]. Herein, we propose a MR-proANP cut-off (660pmol/L) to identify patients at an extremely high risk for death.

Procalcitonin emerged as a diagnostic and prognostic marker of bacterial infection. Recently, several interventional trials proved procalcitonin beneficial for guiding

therapy in lower respiratory tract infections, including VAP [24, 46-49]. Procalcitonin on VAP-onset presented an AUC of 0.712 for predicting survival in our population. AUC values were slightly better on day 3 and 5 after VAP-onset (data not shown). However, since parameters assessed at VAP-onset are statistically more robust and clinically more relevant we focused on the very first day. Nonetheless, our figures are lower than those described by others [20, 21, 50]. Duflo et al. reported an AUC of 0.79 using procalcitonin for predicting VAP associated mortality [20]. However, all patients included in the previous study had three out of three diagnostic parameters. Additionally, only microbiologically diagnosed VAP cases have been analyzed. Therefore, a great proportion of patients meeting fewer diagnostic criteria for VAP might have been missed. Moreover, the selection of cases with severe bacterial infection might have led to an overestimation of the procalcitonin performance. Restricting the population to microbiological confirmed cases of VAP has also increased the predictive property of procalcitonin in our study. However, patient numbers were too small to stratify microbiologically confirmed and unconfirmed cases. It has been proposed that procalcitonin kinetics levels might predict survival in VAP and critical ill patients [22, 23]. In line with those findings, we report a different decline of procalcitonin levels in survivors and non-survivors. Luyt et al. suggested procalcitonin to be strongly related to unfavourable outcome in VAP [21]. The superiority of procalcitonin in the previous study might be explained by more restrictive inclusion criteria and the use of a combined endpoint. Nevertheless, the clinical significance of a combined endpoint including death, VAP recurrence and extrapulmonary infection is greatly diverse. In our trial, we used death as the sole clinical endpoint. The findings show that procalcitonin is a predictor of survival in VAP. However, its value is limited and it is unlikely that procalcitonin sufficiently

predicts survival. Therefore, we do not support a single procalcitonin measurement for risk assessment in VAP.

Disease specific scores, ranging from the Apgar score to the Mini-Mental State Examination, have been verified over decades and remain cornerstones for guiding therapy. In intensive care, due to multiple organ dysfunction, non-disease specific scores are probably more suitable to assess mortality. SAPS II and SOFA are two of the most widespread ICU scoring systems. They are powerful tools to quantify disease severity and estimate mortality [51-54]. Nevertheless, most studies investigating ICU-scores were done to describe the outcome of a patient group as a whole. Thus, the value in an individual patient may be limited [55, 56]. In our study, we could show that both SAPS II and SOFA can successfully predict outcome of individual VAP patients. However, a single MR-proANP measurement had a similar value for predicting survival as scores composed of 6 (SOFA) or 15 (SAPS II) parameters including another sub-score (Glasgow Coma Scale). Therefore, MR-proANP is potentially superior to every of the other 18 parameters included in SOFA and SAPS II. In this context, this is the first study to report that adding biomarkers stepwise improves the performance of clinical scores for survival prediction in VAP. Out of 25 parameters included in our L1-penalized model, MR-proANP was identified as the most important variable, followed by SOFA and SAPS II.

Procalcitonin was clearly inferior to MR-proANP and its additional contribution to ICU-scores and MR-proANP was small. However, both, procalcitonin and MR-proANP, were able to improve ICU-scores, independently. Together, they might comprise a wide spectrum of disease in VAP and intensive care. Thus, we suggest

circulating biomarkers, such as MR-proANP and procalcitonin, to be complementary to ICU-scores such as SAPS II and SOFA.

Practicability and time-efficiency are important requirements for critical care measures. Based on our data, we have suggested an easily applicable nomogram combining MR-proANP and SAPS for survival prediction. Particularly in patients with moderately elevated SAPS II scores, the additional determination of MR-proANP markedly improves risk stratification. Prompt risk stratification has been shown to influence patient management, decrease costs and potentially improves clinical outcome [57-60]. However, clinical impact and costs, particularly in concern to new biomarkers, need to be addressed in future studies.

Our study has several limitations. First, the small sample size may influence discrimination. Thus, our results need to be considered hypothesis-generating and larger prospective trials are essential to validate the findings of this pilot study. Second, the clinical diagnostic criteria for VAP remain a subject of debate. For this reason, we provide results for both clinically and microbiologically diagnosed VAP. Third, cardiac function certainly affects MR-proANP release and survival. Therefore interaction and/or confounding are likely. In a restricted population (patients with echocardiography), neither patients with systolic nor patients with diastolic dysfunction, were at increased risk for death (data not shown). Nevertheless, when analyzing the restricted population, MR-proANP was a strong predictor of survival. Finally, in contrast to SOFA, the use of SAPS II after ICU admission has not been evaluated. Nonetheless, a reassessment of disease severity at the moment of VAP diagnosis is considered to allow a better and more precise stratification of risk and prediction of mortality [45, 61].

In summary, the combination of MR-proANP and procalcitonin improves the diagnostic performance of SAPS II and SOFA in VAP survival. By complementing rather than replacing clinical judgements, the combination of prognostic scores and biomarkers represent a new promising approach for risk stratification in VAP.

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ABBREVIATION LIST:

ARDS	acute respiratory distress syndrome
AUC	area under the curve
BAL	bronchoalveolar lavage
CPIS	clinical pulmonary infection score
EA	endotracheal aspirates
ICU	intensive care unit
IQR	interquartile range
MR-proANP	mid regional – pro atrial natriuretic peptide
ODIN	organ dysfunction and/or infection
p	p-value
PCT	procalcitonin
PSB	protected specimen brush
ROC	receiver operating characteristic
SE	standard error
SAPS II	simplified acute physiologic score II
SOFA	sequential related organ failure assessment
VAP	ventilator associated pneumonia

FIGURE LEGENDS

Figure 1A/B

Time-course of MR-proANP (Figure 1A) and procalcitonin (Figure 1B) during the first 10 days after VAP-onset, comparing survivors (n=81) and non-survivors (n=20); p-values in respect of biomarker trend over time, in survivors and non-survivors.

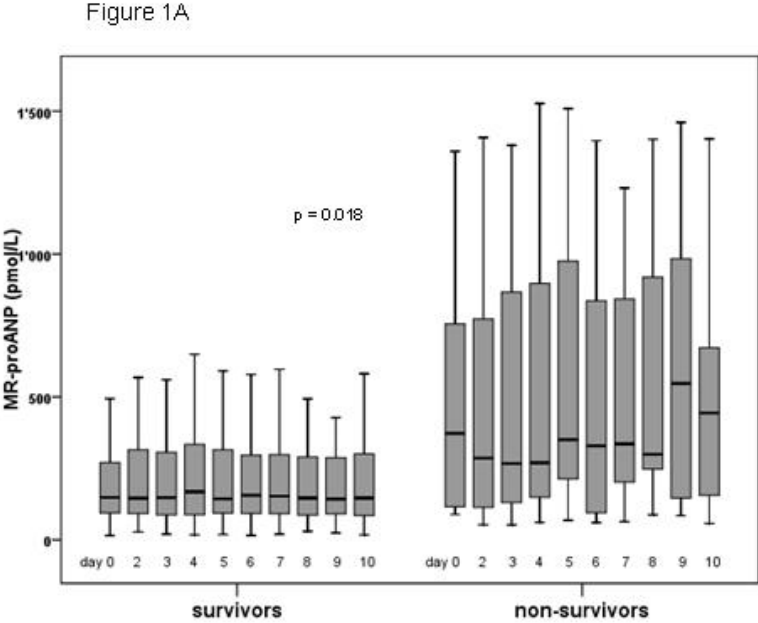


Figure 1B

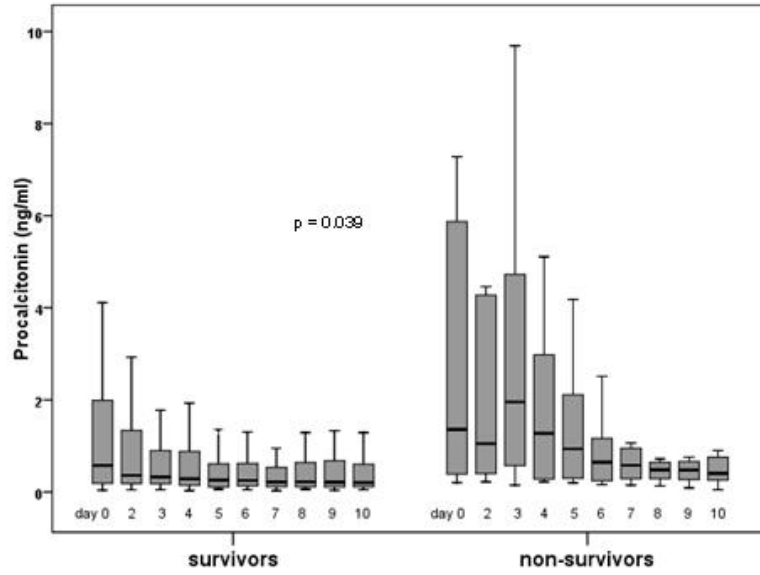


Figure 2

Kaplan-Meier estimates of the survival probability within 28 days of VAP-onset in MR-proANP quartiles.

Figure 2

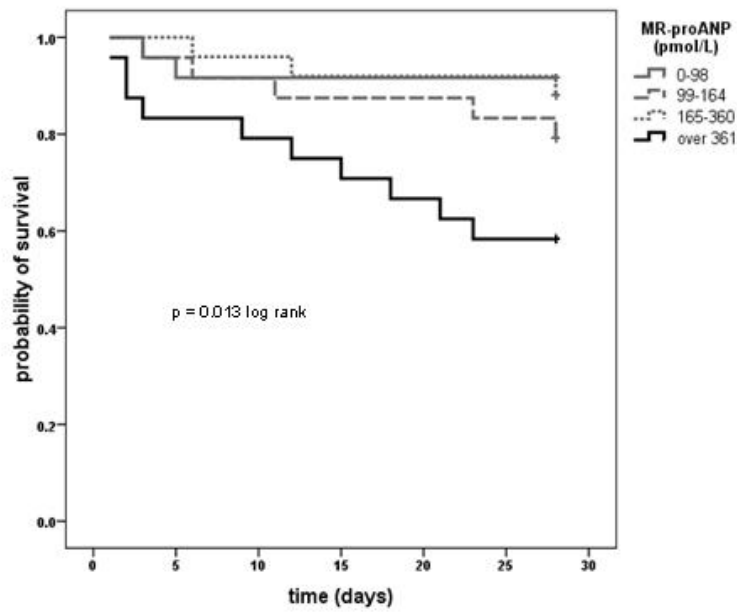


Figure 3A/B

Receiver operating characteristic curves showing a stepwise improvement of SAPS II (Figure 3A) and SOFA (Figure 3B) by adding MR-proANP and procalcitonin (PCT).
Abbreviations: AUC: area under the curve.

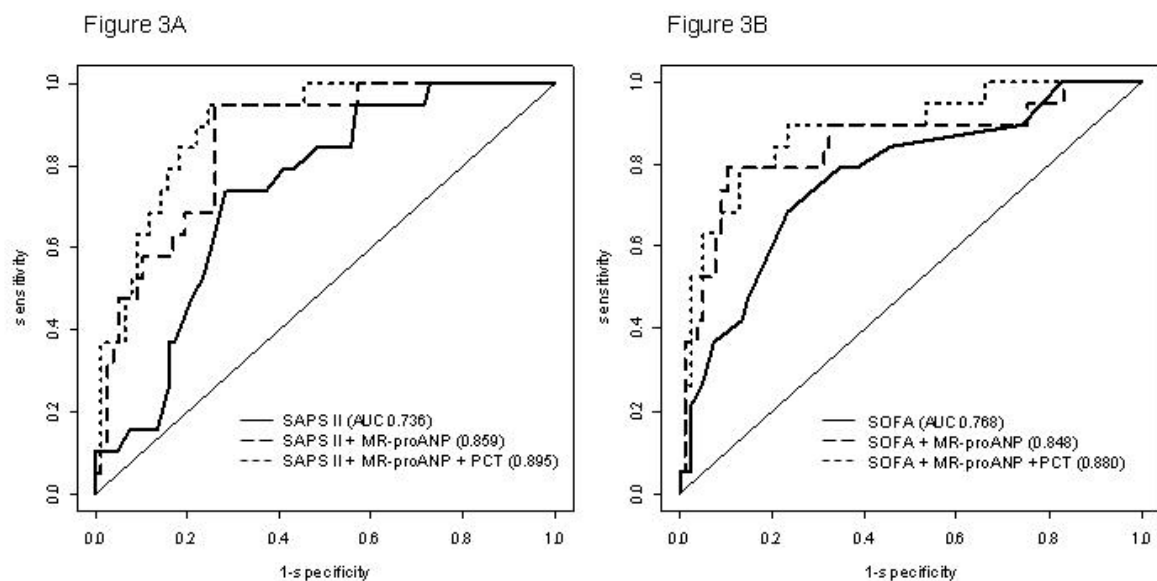


Figure 4

Nomogram to estimate survival, combining SAPS II and MR-proANP, assessed on VAP-onset. MR-proANP adds prognostic information mainly in the group of intermediate SAPS II. The example above shows two patients with an identical SAPS II (47 points). The first patient (survivor, dotted line) with a MR-proANP level of 449pmol/L at VAP-onset, had an estimated risk of death around 30%. Whereas the second patient (non-survivor, dashed line) with a MR-proANP level of 1360pmol/L had a risk exceeding 80%.

Figure 4

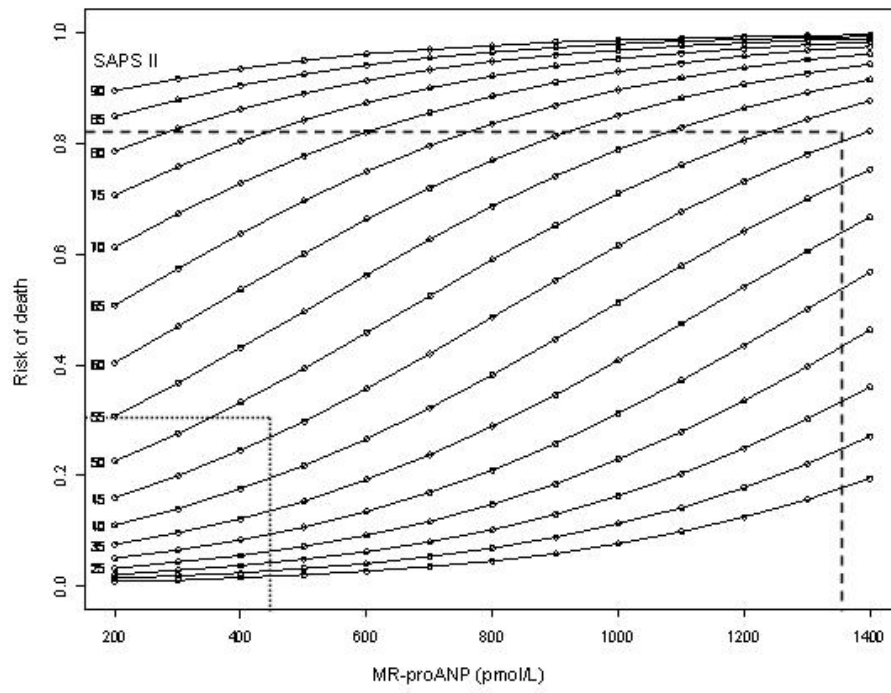


Table 1: Demographics of 101 patients at VAP-onset

	Total n=101	Survivors n=81	non-survivors n=20	p-value
Age - years	57 [43-70]	55 [42-68]	67 [52-75]	0.033
Gender – male	74 (74)	61 (76)	13 (65)	0.459
Admission category				
Medical	53 (53)	41 (51)	12 (60)	0.615
Elective surgery	4 (4)	4 (5)	0 (0)	0.582
Emergency surgery	43 (43)	35 (43)	8 (40)	0.994
Coexisting illnesses				
Cardiac	48 (48)	37 (46)	11 (55)	0.619
Pulmonary	19 (19)	14 (17)	5 (25)	0.523
Renal	16 (16)	11 (14)	5 (25)	0.302
Hematological/ oncological	8 (8)	4 (5)	4 (20)	0.047
Reason for mechanical ventilation				
Cardiovascular failure	32 (32)	25 (31)	7 (35)	0.930
Acute respiratory failure	53 (53)	42 (52)	11 (55)	0.998
Trauma	33 (33)	28 (35)	5 (25)	0.582
Neurologic failure	30 (30)	26 (32)	4 (20)	0.431
Sepsis	25 (25)	22 (27)	3 (15)	0.387
Miscellaneous	10 (10)	9 (11)	1 (5)	0.682
Duration of mechanical ventilation before VAP – days	6 [3.5-9]	6 [3-9]	5.5 [4-9.8]	0.801
Antibiotics within 14 days before VAP-onset	76 (75)	59 (73)	17 (85)	0.387
Vital parameters at VAP-onset				
Heart Rate - per min	90 [79-103]	90 [80-110]	88 [76-95]	0.344
MAP - mmHg	77 [70-90]	77 [70-90]	75 [68-85]	0.436
Temperature - °C	38.0 [37.4-38.6]	38.1 [37.6-38.8]	37.4 [37.0-38.3]	0.010
PaO ₂ /FIO ₂	188 [135-253]	192 [140-254]	173 [126-225]	0.290
SaO ₂ - %	97 [94-99]	97 [94-99]	98 [96-99]	0.468
WBC - 10 ⁹ /L	11.6 [8.0-15.0]	11.2 [8.0-14.6]	13.3 [9.3-18.0]	0.209
Microbiology				
Positive microbiological cultures (EA, BAL, PSB)	74 (76)	58 (73)	16 (89)	0.226
Positive blood cultures	34 (34)	29 (36)	5 (25)	0.515
Clinical Scores at VAP-onset				
SAPS II	40.5 [32.3-51.0]	38.0 [31.0-47.0]	48.0 [42.0-55.0]	0.002
ODIN - score	2 [1-3]	2 [1-2]	3 [1-4]	0.050
SOFA - score	7.0 [6.0-9.8]	6 [5-9]	9 [7-14]	0.002
CPIS	7.5 [6.0-9.0]	8.0 [6.0-9.0]	7.0 [6.0-8.0]	0.799
Biomarkers at VAP-onset				
MR-proANP - pmol/L	163 [98-374]	149 [93-278]	373 [114-784]	0.003
Procalcitonin - ng/mL	0.69 [0.22-2.34]	0.58 [0.19-2.00]	1.36 [0.38-6.04]	0.017

Values are given as numbers (%) or median [IQR]. Fever was defined as a body temperature above 38°C/ 100.4°F; leukocytosis/-penia as a leukocyte count higher than 11000/ μ L or lower than 3000/ μ L;

Abbreviations: MAP: mean arterial pressure; PaO₂/FIO₂: ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen; SaO₂: oxygen saturation; WBC: leukocyte count; EA: endotracheal aspirates; BAL: bronchoalveolar lavage; PSB: protected specimen brush; SAPS II: simplified acute physiology score II; ODIN: organ dysfunction and/or infection; SOFA: sequential organ failure assessment; CPIS: clinical pulmonary infection score.

Table 2: Spearman correlation of biomarkers with age and vital parameters

	MR-proANP		Procalcitonin	
	Spearman's rho	p-value	Spearman's rho	p-value
Age	0.62	<0.001	0.19	0.066
Vital parameters				
Heart rate	0.23	0.024	0.11	0.280
MAP	0.30	0.003	0.18	0.071
Temperature	0.14	0.161	0.06	0.530
PaO ₂ /FiO ₂	0.07	0.505	0.02	0.819
SaO ₂	0.03	0.774	0.22	0.041
WBC	0.17	0.094	0.16	0.109

Abbreviations: MAP: mean arterial pressure; PaO₂/FIO₂: ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen; SaO₂: oxygen saturation; WBC: leukocyte count.

Table 3: Association of biomarkers with gender and comorbidities

	MR-proANP p-value	Procalcitonin p-value
Gender	0.890	0.037
Coexisting illness		
Cardiac	<0.001	0.276
Pulmonary	0.014	0.960
Renal	<0.001	0.003
Hematological/ oncological	0.054	0.627