Eur Respir J 2011; 37: 595–603
DOI: 10.1183/09031936.00023810


# Midregional pro-atrial natriuretic peptide and procalcitonin improve survival prediction in VAP

**L. Boeck*, P. Eggimann#, N. Smyrnios¶, H. Pargger+, N. Thakkar¶, M. Siegemund+, S. Marsch§, J. Rakic*, M. Tamm* and D. Stolz*,f**

**ABSTRACT: Ventilator-associated pneumonia (VAP) 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 optimised over the last decades. Recently, new biomarkers have gained interest in the prognostic field. We assessed whether midregional pro-atrial natriuretic peptide (MR-proANP) and procalcitonin (PCT) improve the predictive value of the Simplified Acute Physiologic Score (SAPS) II and Sequential Related Organ Failure Assessment (SOFA) in VAP.**

**Specified end-points of a prospective multinational trial including 101 patients with VAP were analysed. Death <28 days after VAP onset was the primary end-point.**

**MR-proANP and PCT were elevated at the onset of VAP in nonsurvivors compared with 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 VAP 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 PCT to SAPS II and SOFA improved their predictive properties (area under the curve 0.895 and 0.880).**

**We conclude that the combination of two biomarkers, MR-proANP and PCT, improve survival prediction of clinical severity scores in VAP.**

**KEYWORDS: Biomarker, midregional pro-atrial natriuretic peptide, procalcitonin, prognosis, risk stratification, ventilator-associated pneumonia**

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 exceed US$ 40,000 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 therapeutic measures [5]. In this context, different clinical presentations, ranging from subtle to very obvious, combined with severe underlying diseases, make risk stratification in VAP particularly challenging. Several clinical severity scores, such as the Acute Physiology and Chronic Health Evaluation (APACHE), Simplified Acute Physiological Score (SAPS) and Sequential Related Organ Failure Assessment (SOFA), have been 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 (pro-ANP, amino acids 1–98), a stable fragment of the atrial natriuretic peptide (ANP) precursor, is secreted in in equimolar quantities with 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]. N-terminal 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 nonsurvivors has been assessed in

**For editorial comments see page 486.**

AFFILIATIONS
*Clinic of Pulmonary Medicine and Respiratory Cell Research, +Division of Anesthesiology and Surgical Intensive Care Medicine, §Division of Medical Intensive Care Medicine, University Hospital, Basel, #Dept of Adult Critical Care Medicine, University Hospital, Lausanne, Switzerland. ¶Division of Pulmonary, Allergy and Critical Care Medicine, UMass Memorial Medical Center, Worcester, MA, and fHarvard School of Public Health, Boston, MA, USA.

CORRESPONDENCE
D. Stolz
University Hospital
Petersgraben 4
CH-4031 Basel
Switzerland
E-mail: stolzd@uhbs.ch

Received:
Feb 11 2010
Accepted after revision:
May 25 2010
First published online:
June 07 2010

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

respiratory tract infections including pneumonia, sepsis and VAP [12–15].

Procalcitonin (PCT) 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, PCT also provides prognostic information [7, 17–19]. Accordingly, high and increasing PCT levels were independent predictors of death in critically 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 haemodynamic (midregional (MR)-proANP) and the infectious (PCT) consequences of VAP, could add to the predictive value of clinical severity scores. We analysed data of a well-characterised cohort of 101 patients with clinically diagnosed VAP. The primary end-point was death <28 days after VAP onset.

## MATERIALS AND METHODS

### Setting and study population

Data from a prospective multicentre trial including 101 patients with clinically diagnosed VAP were analysed [24]. In brief, the main objective of the study was to evaluate PCT-guided antibiotic de-escalation in VAP compared to usual care. The study took place in seven medical and surgical intensive care units (ICUs) (UMass Memorial Medical Center, Worcester, MA, USA; University Hospital Lausanne, Lausanne, Switzerland; and University Hospital Basel, Basel, Switzerland). The analysis of prognostic predictors in the study population was a predefined 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 (identifier number ISRCTN61015974). Written informed consent was obtained from all included patients or their legal representatives.

### Diagnostic criteria

Diagnosis of VAP was established using 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 out of the following: purulent tracheal secretions, fever (body temperature >38°C (100.4°F)), and leukocytosis or leukopenia (leukocyte count >11,000 or <3,000 cells·μL$^{-1}$, respecitively). ICU patients were eligible for the study if they met all the following criteria: 1) intubated for mechanical ventilation for ⩾48 h; 2) >18 yrs; and 3) clinically 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 (>0.5 mg·kg$^{-1}$·day$^{-1}$ for >1 month); 4) were immunosuppressed; or 5) had a coexisting extrapulmonary infection diagnosed in the first 3 days and requiring antibiotic therapy for ⩽3 days. Microbiologically confirmed VAP was defined by a significant growth of quantitative cultures of endotracheal aspirates, bronchoalveolar lavage or protected specimen brush specimens [1].

### Baseline assessment and follow-up

At the time of enrolment, the following information was recorded from each subject: age, sex, pre-existing 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), arterial oxygen saturation ($S_{a,O_2}$), arterial oxygen tension ($P_{a,O_2}$)/inspiratory oxygen fraction ($F_{I,O_2}$) ratio, leukocyte count (white blood cells; WBCs), and MR-proANP and PCT serum levels. The following indices were calculated: SAPS II, SOFA score, Organ Dysfunction and/or Infection (ODIN) score and Clinical Pulmonary Infection Score (CPIS). During the 28-day follow-up period, the following information was recorded: body temperature; heart rate; MAP; $S_{a,O_2}$; $P_{a,O_2}/F_{I,O_2}$; WBCs; SOFA, ODIN and CPIS; mechanical ventilation status; antibiotic use; and survival throughout the 28-day study period. Serum MR-proANP and PCT levels were determined at VAP onset and daily for 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 nonsurvivors; all others were classified as survivors. No patient was lost to follow-up.

### MR-proANP and PCT measurements

MR-proANP measurements were performed with 50 μL serum using a test based on the time-resolved amplified cryptate emission (TRACE) technology (MR-proANP KRYPTOR; BRAHMS AG; Hennigsdorf, Germany). The lower detection limit of the assay is 6 pmol·L$^{-1}$ and the functional assay sensitivity (defined as the lowest value with an interassay coefficient of variation <20%) is 23 pmol·L$^{-1}$ [9]. The median MR-proANP level of healthy subjects was 45 pmol·L$^{-1}$. PCT was measured in 50 μL serum using TRACE technology (PCT sensitive KRYPTOR; BRAHMS AG; Hennigsdorf, Germany). A lower detection limit of 0.02 ng·mL$^{-1}$ and a functional assay sensitivity of 0.06 ng·mL$^{-1}$ were identified [26].

### Statistical analyses

Discrete variables are presented as n (%) and continuous variables as median (interquartile range (IQR)). Comparability of groups was analysed by Chi-squared test, Fisher's exact test or Mann–Whitney U-test, as appropriate. Correlation analyses were performed using Spearman's ρ. To detect the time-course of the biomarkers across survivors and nonsurvivors, a linear mixed-effect model with the 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-transformed 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 analysed by Kaplan–Meier survival curves and compared by the log rank test. Area under the curve (AUC) values for receiver operating characteristic (ROC) 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 are presented as mean ± SE. In order to predict survival, an L1-penalised logistic regression model was performed. This is an adaptation of Lasso regression to generalised linear models [28]. This approach is justified when

the number of predictors is large compared with 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 (R Development Core Team, Vienna, Austria). All tests were two-tailed; p-values of $<0.05$ were defined as significant. Data were analysed using statistical software (Statistical Package for Social Sciences version 16 for Windows; SPSS, Chicago IL, USA).

## RESULTS

### *Baseline characteristics*

A total of 101 patients were included in the study. Detailed baseline characteristics for survivors and nonsurvivors are summarised in table 1. The median age was 57 yrs (IQR

**TABLE 1** Demographics of 101 patients at the onset of ventilator-associated pneumonia (VAP)

| | All | Survivors | Nonsurvivors | p-value |
|---|---|---|---|---|
| **Patients n** | 101 | 81 | 20 | |
| **Age yrs** | 57 (43–70) | 55 (42–68) | 67 (52–75) | **0.033** |
| **Males** | 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 |
| Haematological/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 beats·min$^{-1}$ | 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** |
| $Pa,O_2/Fi,O_2$ | 188 (135–253) | 192 (140–254) | 173 (126–225) | 0.290 |
| $Sa,O_2$ % | 97 (94–99) | 97 (94–99) | 98 (96–99) | 0.468 |
| WBCs $\times 10^9$ cells·L$^{-1}$ | 11.6 (8.0–15.0) | 11.2 (8.0–14.6) | 13.3 (9.3–18.0) | 0.209 |
| **Microbiology** | | | | |
| Positive EA, BAL or PSB cultures | 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 | 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$^{-1}$ | 163 (98–374) | 149 (93–278) | 373 (114–784) | **0.003** |
| PCT ng·mL$^{-1}$ | 0.69 (0.22–2.34) | 0.58 (0.19–2.00) | 1.36 (0.38–6.04) | **0.017** |

Data are presented as median (interquartile range) or n (%), unless otherwise stated. Fever was defined as a body temperature >38°C (100.4°F). Leukocytosis and leukopenia were defined as a leukocyte count >11,000 and <3,000 cells·μL$^{-1}$, respectively. MAP: mean arterial pressure; $Pa,O_2$: arterial oxygen tension; $Fi,O_2$: inspiratory oxygen fraction; $Sa,O_2$: arterial oxygen saturation; WBC: white blood cells; EA: endotracheal aspirates; BAL: bronchoalveolar lavage; PSB: protected specimen brush; SAPS: Simplified Acute Physiology Score; ODIN: Organ Dysfunction and/or Infection; SOFA: Sequential Organ Failure Assessment; CPIS: Clinical Pulmonary Infection Score; MR-proANP: midregional pro-atrial natriuretic peptide; PCT: procalcitonin. Bold indicates statistically significant p-values.

43–70 yrs). Cardiac comorbidities were reported as follows: arterial hypertension (n=31), coronary artery disease (n=30), myocardial infarction (n=17), cardiac arrhythmia (n=16), congestive heart failure (n=11) and aortic aneurysm (n=5). Microbiological analyses of respiratory tract secretions identified a causative organism in 74 (76%) patients. 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 β-lactam or an antipseudomonal carbapenem, was applied in 86% of cases. 20 patients died during the study period. Deaths were due to traumatic brain injury/subarachnoid hemorrhage (n=8), respiratory failure/acute respiratory distress syndrome (n=5), septic shock (n=3), cardiogenic shock (n=2), multiorgan failure (n=1) and acute liver failure (n=1).

### MR-proANP in VAP

At VAP onset, median MR-proANP was 163 pmol·L$^{-1}$ (IQR 98–374 pmol·L$^{-1}$). Elevated MR-proANP was associated with age, heart rate, MAP, and the presence of cardiac, pulmonary and renal comorbidities (tables 2 and 3). MR-proANP was significantly elevated in nonsurvivors (median 373 pmol·L$^{-1}$ (IQR 114–784 pmol·L$^{-1}$) *versus* 149 pmol·L$^{-1}$ (IQR 93–278) pmol·L$^{-1}$; 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 nonsurvivors (p=0.018; fig. 1a). Kaplan–Meier survival function was significantly different across MR-proANP quartiles at VAP onset (log rank p=0.013; fig. 2). In ROC analysis, the mean±SE AUC of MR-proANP at VAP onset to predict mortality was 0.801±0.062. An MR-proANP threshold of 660 pmol·L$^{-1}$ had the highest accuracy for predicting death. The associated sensitivity and specificity were 45% and 97%, respectively. Accordingly, the positive likelihood ratio was 17.3. We assessed a positive and negative predictive value of 82% and 87%. The use of the 660-pmol·L$^{-1}$ MR-proANP cut-off yielded an odds ratio of 30.7 (95% CI 5.9–161.0) for predicting death at VAP onset.

### PCT in VAP

Median PCT on VAP onset was 0.69 ng·mL$^{-1}$ (IQR 0.22–2.34 ng·mL$^{-1}$) and significantly correlated with $S_{a,O_2}$, renal disease and sex (tables 2 and 3). Age, and cardiac and pulmonary comorbidities did not influence PCT levels. PCT levels were significantly elevated in nonsurvivors (1.36 ng·mL$^{-1}$ (IQR 0.38–6.04 ng·mL$^{-1}$) *versus* 0.58 ng·mL$^{-1}$ (IQR 0.19–2.00 ng·mL$^{-1}$); 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; fig. 1b). The median relative decrease of PCT within 72 h after VAP onset was 26% in survivors and 7% in nonsurvivors. PCT quartiles did not differ in survival (log rank p=0.076). The mean±SE AUC for PCT on VAP onset to predict mortality was 0.712±0.069.

### Combination of MR-proANP, PCT and ICU scores

At VAP onset, the mean±SE AUCs of SAPS II and SOFA to predict 28-day mortality were 0.736±0.067 and 0.768±0.065, respectively (fig. 3a and b). 25 parameters, such as age, vital signs, laboratory values, comorbidities, SAPS II, SOFA, ODIN, and PCT and MR-proANP serum levels at VAP onset were included in an L1-penalised logistic regression model analysing 28-day survival. MR-proANP was identified as the best predictor of survival, followed by SOFA and SAPS II. Adding MR-proANP at VAP onset to clinical scores significantly improved the AUC for SAPS II to 0.859±0.055 (p=0.024). The AUC of SOFA plus MR-proANP was enhanced to 0.848±0.056 (p=0.095). A further refinement of the model was achieved by including PCT at VAP onset. SAPS II in conjunction with MR-proANP and PCT significantly improved survival prediction compared with SAPS II alone (AUC 0.895±0.048; p=0.003; fig. 3a). Similarly, predictive properties of SOFA increased by adding MR-proANP and PCT (AUC 0.880±0.051; p=0.087; fig. 3b). In order to refine risk estimation in a clinically feasible fashion, we designed a two-dimensional diagram for risk stratification in VAP (fig. 4). In this nomogram, MR-proANP and SAPS II assessed at VAP

**TABLE 2** Spearman correlation of biomarkers with age and vital parameters

| | MR-proANP | | PCT | |
|---|---|---|---|---|
| | Spearman's ρ | p-value | Spearman's ρ | 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 |
| $P_{a,O_2}/F_{I,O_2}$ | 0.07 | 0.505 | 0.02 | 0.819 |
| $S_{a,O_2}$ | 0.03 | 0.774 | 0.22 | **0.041** |
| WBCs | 0.17 | 0.094 | 0.16 | 0.109 |

MR-proANP: midregional pro-atrial natriuretic peptide; PCT: procalcitonin; MAP: mean arterial pressure; $P_{a,O_2}$: arterial oxygen tension; $F_{I,O_2}$: inspiratory oxygen fraction; $S_{a,O_2}$: arterial oxygen saturation; WBCs: white blood cells. Bold indicates statistically significant p-values.

**TABLE 3** Association of biomarkers with sex and comorbidities

| | p-value | |
|---|---|---|
| | MR-proANP | PCT |
| **Sex** | 0.890 | **0.037** |
| **Coexisting illness** | | |
| Cardiac | **<0.001** | 0.276 |
| Pulmonary | **0.014** | 0.960 |
| Renal | **<0.001** | **0.003** |
| Haematological/oncological | 0.054 | 0.627 |

MR-proANP: midregional pro-atrial natriuretic peptide; PCT: procalcitonin. Bold indicates statistically significant p-values.

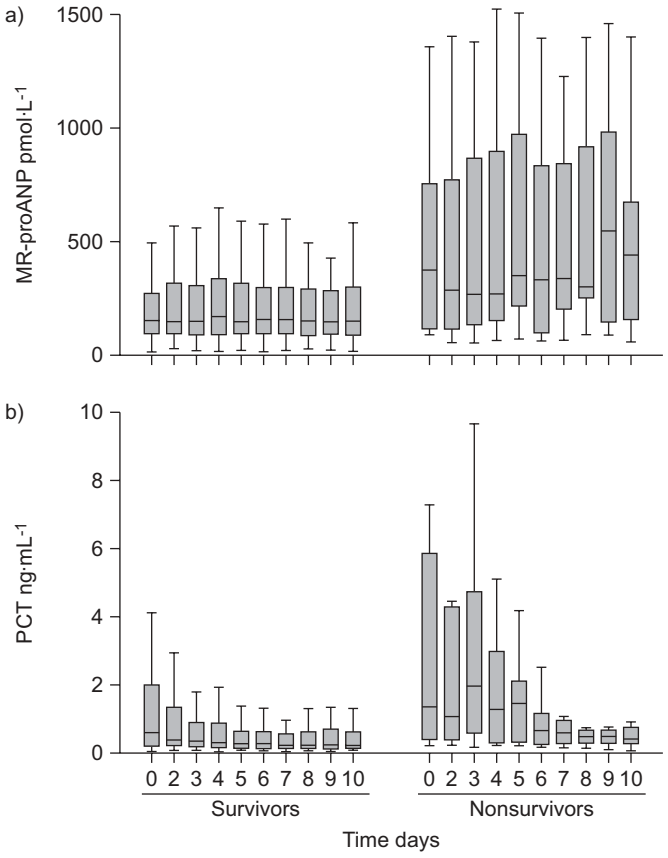


**FIGURE 1.** Time-course of a) midregional pro-atrial natriuretic peptide (MR-proANP; p=0.018) and b) procalcitonin (PCT; p=0.039) during the first 10 days after ventilator-associated pneumonia onset, comparing survivors (n=81) and nonsurvivors (n=20); p-values are in respect of biomarker trend over time, in survivors and nonsurvivors.

onset predict the risk of death within the following 28 days. PCT did not provide useful additional information.

The predictive values of MR-proANP, PCT and clinical scores were also analysed in the subgroup of patients with microbiologically confirmed VAP (n=74). Compared with patients with clinically diagnosed VAP, the AUC for PCT at VAP onset to predict mortality within 28 days was higher in patients with microbiologically diagnosed VAP (mean±SE AUC 0.767±0.065 *versus* 0.712±0.065). In contrast, the predictive property of MR-proANP at VAP onset was similar in both populations (AUC 0.820±0.060 *versus* 0.801±0.060). Combining MR-proANP with SOFA or SAPS II enhanced AUC values compared with the single score (SOFA AUC 0.848±0.056, p=0.071; SAPS II AUC 0.906±0.046, p=0.019). The conjunction of MR-proANP, PCT and SOFA at VAP onset predicted survival with a significantly better accuracy compared with the SOFA score (AUC 0.900±0.047; p=0.032).

## DISCUSSION

We report three main findings. First, circulating MR-proANP and PCT levels at VAP onset are significantly elevated in nonsurvivors compared with survivors. Secondly, a single MR-proANP determination at VAP onset is a suitable marker for survival prediction. Finally, the combination of MR-proANP

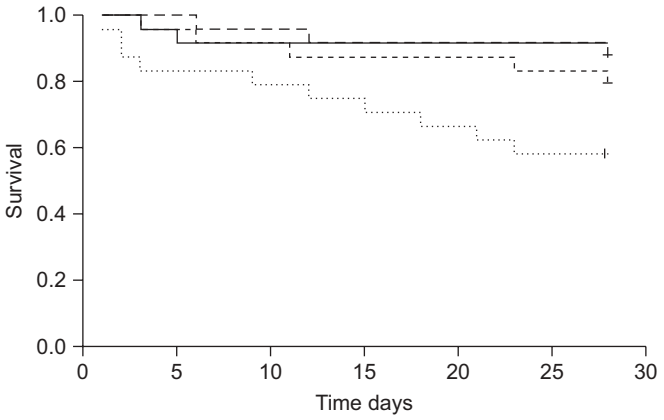


**FIGURE 2.** Kaplan–Meier estimates of the survival probability within 28 days of ventilator-associated pneumonia onset in midregional pro-atrial natriuretic peptide quartiles of 0–98 (——), 99–164 (-----), 165–360 (– – –) and >361 pmol·L$^{-1}$ (·····). Log rank p=0.013.

and PCT improves survival prediction of SAPS II and SOFA, two well established ICU scores.

Circulating natriuretic peptides, such as ANP and brain natriuretic peptide, 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 the ICU has been challenged [32]. However, natriuretic peptides have 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 major stimuli for ANP release. Renal failure elevates proANP levels, most probably 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 tumour necrosis factor (TNF)-α was associated with increased ANP independently of left ventricular function [44]. This indicates that TNF-α might exert direct cardiotoxic effects or stimulate ANP release. Therefore, it is tempting to hypothesise that in inflammatory conditions such as VAP, ANP secretion is partially mediated through cytokine release.

We have shown that MR-proANP is elevated in nonsurvivors and that the slope of decline differs significantly between survivors and nonsurvivors in VAP. Our results are consistent with the findings reported by SELIGMAN *et al.* [15] suggesting MR-proANP to be associated with mortality and the severity of

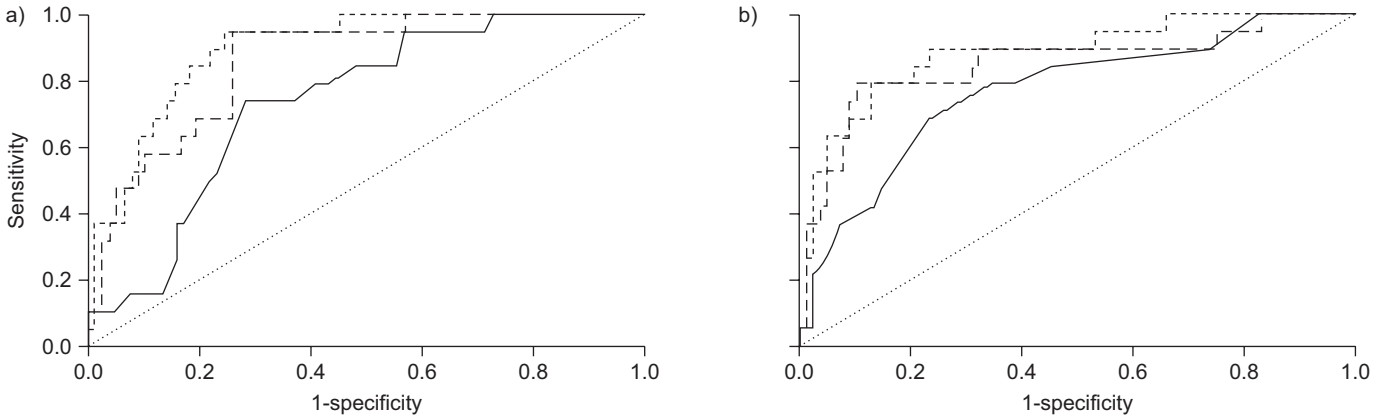


**FIGURE 3.** Receiver operating characteristic curves showing a stepwise improvement of a) Simplified Acute Physiologic Score (SAPS) II (—: SAPS II, area under the curve (AUC) 0.736; – – –: SAPS II plus midregicnal pro-atrial natriuretic peptide (MR-proANP), AUC 0.859; -----: SAPS II plus MR-proANP plus procalcitonin (PCT), AUC 0.895) and b) Sequential Related Organ Failure Assessment (SOFA) (—: SOFA, AUC 0.768; – – –: SOFA plus MR-proANP, AUC 0.848; ---: SOFA plus MR-proANP plus PCT, AUC 0.880) by adding MR-proANP and PCT.

sepsis in a VAP cohort. Notably, that study has restricted the analysis to VAP patients presenting a clinical pulmonary infection score above six on day 3 and analysed proANP levels only at two time-points (day 0 and 4). In our cohort, MR-proANP levels were persistently increased over the 10 days following VAP onset in nonsurvivors. We report a similar mortality prediction of proANP in VAP compared with BERDAL *et al*. [35], who assessed 70 unselected mechanically ventilated, critically ill patients. Thus, MR-proANP seems to reflect multiple comorbidities and infectious conditions related to survival in intensive care. Despite the exceptionally high mortality and resource utilisation in VAP, specific tools assessing mortality are scarce [45]. Herein, we propose a MR-proANP cut-off (660 pmol·L$^{-1}$) to identify patients at an extremely high risk for death.

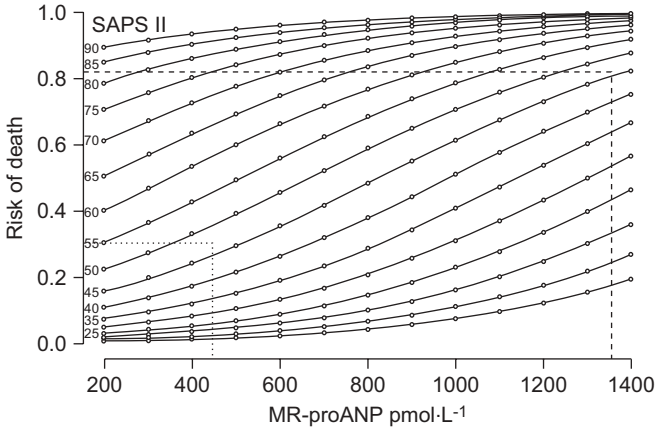


**FIGURE 4.** Nomogram to estimate survival, combining Simplified Acute Physiologic Score (SAPS) II and midregicnal pro-atrial natriuretic peptide (MR-proANP) assessed at ventilator-associated pneumonia (VAP) onset. MR-proANP adds prognostic information mainly in the group of intermediate SAPS II. Two patients with an identical SAPS II (47 points) are shown. The first patient (survivor; ·····), with a MR-proANP level of 449 pmol·L$^{-1}$ at VAP onset, had an estimated risk of death around 30%; the second patient (nonsurvivor; -----), with a MR-proANP level of 1,360 pmol·L$^{-1}$, had a risk >80%.

PCT first emerged as a diagnostic and prognostic marker of bacterial infection. Recently, several interventional trials showed PCT was beneficial for guiding therapy in lower respiratory tract infections, including VAP [24, 46–49]. PCT at 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.* [20] reported an AUC of 0.79 using PCT for predicting VAP-associated mortality. However, all patients included in their study met three out of three diagnostic parameters. Additionally, only microbiologically diagnosed VAP cases were analysed. 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 lead to an over-estimation of the PCT performance. Restricting the population to microbiologically confirmed cases of VAP has also increased the predictive property of PCT in our study. However, patient numbers were too small to stratify microbiologically confirmed and unconfirmed cases. It has been proposed that PCT kinetics levels might predict survival in VAP and critical ill patients [22, 23]. Consistent with those findings, we report a different decline of PCT levels in survivors and nonsurvivors. LUYT *et al.* [21] suggested PCT to be strongly related to unfavourable outcome in VAP. The superiority of PCT in the previous study might be explained by more restrictive inclusion criteria and the use of a combined end-point. Nevertheless, the clinical significance of a combined end-point including death, VAP recurrence and extrapulmonary infection is greatly diverse. In our trial, we used death as the sole clinical end-point. The findings show that PCT is a predictor of survival in VAP. However, its value is limited and it is unlikely that PCT sufficiently predicts survival. Therefore, we do not support a single PCT 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, nondisease-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 for quantifying disease severity and estimating mortality [51–54]. Nevertheless, most studies investigating ICU scores were performed 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 showed that both SAPS II and SOFA can successfully predict the outcome of individual VAP patients. However, a single MR-proANP measurement had a similar value for predicting survival as scores composed of six (SOFA) or 15 (SAPS II) parameters, including another sub-score (Glasgow Coma Scale). Therefore, MR-proANP is potentially superior to every one of the 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-penalised model, MR-proANP was identified as the most important variable, followed by SOFA and SAPS II.

PCT was clearly inferior to MR-proANP, and its additional contribution to ICU scores and MR-proANP was small. However, both PCT 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 PCT, 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. Especially 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 improve clinical outcome [57–60]. However, clinical impact and costs, particularly with regard 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. Secondly, the clinical diagnostic criteria for VAP remain a subject of debate. For this reason, we provide results for both clinically and microbiologically diagnosed VAP. Thirdly, 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 those with diastolic dysfunction were at increased risk for death (data not shown). Nevertheless, when analysing 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 PCT 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 and promising approach for risk stratification in VAP.

## SUPPORT STATEMENT

D. Stolz was supported by grants from the Swiss National Foundation (PP00P3_128412/1), the Liechtenstein Foundation and the ''Freiwillige Akademische Gesellschaft Basel''. N. Thakkar was supported by the Will Rogers Foundation. Additional funding was granted by the Clinic of Pulmonary Medicine, University Hospital Basel, Basel, Switzerland.

## CLINICAL TRIAL

This study was registered in the Current Controlled Trials Database as ''ProVAP''-Study (identifier number ISRCTN61015974).

## STATEMENT OF INTEREST

Statements of interest for D. Stolz and the study itself can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

## ACKNOWLEDGEMENTS

We thank A. Meyer (Clinic of Pulmonary Medicine and Respiratory Cell Research, University Hospital, Basel, Switzerland), S. Heard, J. Longtine and K. Longtine (all Division of Anesthesiology, UMass Memorial Medical Center, Worcester, MA, USA) for data supply and most helpful support during the study and A. Schötzau (Schötzau & Simmen Statistical Consulting, Basel) for statistical advice.

## REFERENCES

1 American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005; 171: 388–416.
2 Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2002; 165: 867–903.
3 Melsen WG, Rovers MM, Bonten MJ. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. *Crit Care Med* 2009; 37: 2709–2718.
4 Rello J, Ollendorf DA, Oster G, *et al.* Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. *Chest* 2002; 122: 2115–2121.
5 Kollef MH. The prevention of ventilator-associated pneumonia. *N Engl J Med* 1999; 340: 627–634.
6 Gursel G, Demirtas S. Value of APACHE II, SOFA and CPIS scores in predicting prognosis in patients with ventilator-associated pneumonia. *Respiration* 2006; 73: 503–508.
7 Stolz D, Christ-Crain M, Morgenthaler NG, *et al.* Copeptin, C-reactive protein, and procalcitonin as prognostic biomarkers in acute exacerbation of COPD. *Chest* 2007; 131: 1058–1067.
8 Christ-Crain M, Stolz D, Jutla S, *et al.* Free and total cortisol levels as predictors of severity and outcome in community-acquired pneumonia. *Am J Respir Crit Care Med* 2007; 176: 913–920.
9 Morgenthaler NG, Struck J, Thomas B, *et al.* Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin Chem* 2004; 50: 234–236.
10 von Haehling S, Jankowska EA, Morgenthaler NG, *et al.* Comparison of midregional pro-atrial natriuretic peptide with N-terminal pro-B-type natriuretic peptide in predicting survival in patients with chronic heart failure. *J Am Coll Cardiol* 2007; 50: 1973–1980.
11 Laukkanen JA, Kurl S, Ala-Kopsala M, *et al.* Plasma N-terminal fragments of natriuretic propeptides predict the risk of cardiovascular events and mortality in middle-aged men. *Eur Heart J* 2006; 27: 1230–1237.

12 Brueckmann M, Huhle G, Lang S, *et al.* Prognostic value of plasma N-terminal pro-brain natriuretic peptide in patients with severe sepsis. *Circulation* 2005; 112: 527–534.
13 Muller B, Suess E, Schuetz P, *et al.* Circulating levels of pro-atrial natriuretic peptide in lower respiratory tract infections. *J Intern Med* 2006; 260: 568–576.
14 Kruger S, Papassotiriou J, Marre R, *et al.* Pro-atrial natriuretic peptide and pro-vasopressin to predict severity and prognosis in community-acquired pneumonia: results from the German competence network CAPNETZ. *Intensive Care Med* 2007; 33: 2069–2078.
15 Seligman R, Papassotiriou J, Morgenthaler NG, *et al.* Prognostic value of midregional pro-atrial natriuretic peptide in ventilator-associated pneumonia. *Intensive Care Med* 2008; 34: 2084–2091.
16 Muller B, White JC, Nylen ES, *et al.* Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. *J Clin Endocrinol Metab* 2001; 86: 396–404.
17 Gendrel D, Bohuon C. Procalcitonin as a marker of bacterial infection. *Pediatr Infect Dis J* 2000; 19: 679–687.
18 Stolz D, Stulz A, Muller B, *et al.* BAL neutrophils, serum procalcitonin, and C-reactive protein to predict bacterial infection in the immunocompromised host. *Chest* 2007; 132: 504–514.
19 Huang DT, Weissfeld LA, Kellum JA, *et al.* Risk prediction with procalcitonin and clinical rules in community-acquired pneumonia. *Ann Emerg Med* 2008; 52: 48–58.
20 Duflo F, Debon R, Monneret G, *et al.* Alveolar and serum procalcitonin: diagnostic and prognostic value in ventilator-associated pneumonia. *Anesthesiology* 2002; 96: 74–79.
21 Luyt CE, Guerin V, Combes A, *et al.* Procalcitonin kinetics as a prognostic marker of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2005; 171: 48–53.
22 Seligman R, Meisner M, Lisboa TC, *et al.* Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. *Crit Care* 2006; 10: R125.
23 Jensen JU, Heslet L, Jensen TH, *et al.* Procalcitonin increase in early identification of critically ill patients at high risk of mortality. *Crit Care Med* 2006; 34: 2596–2602.
24 Stolz D, Smyrnios N, Eggimann P, *et al.* Procalcitonin for reduced antibiotic exposure in ventilator-associated pneumonia: a randomised study. *Eur Respir J* 2009; 34: 1364–1375.
25 Fabregas N, Ewig S, Torres A, *et al.* Clinical diagnosis of ventilator associated pneumonia revisited: comparative validation using immediate post-mortem lung biopsies. *Thorax* 1999; 54: 867–873.
26 Snider RH Jr, Nylen ES, Becker KL. Procalcitonin and its component peptides in systemic inflammation: immunochemical characterization. *J Investig Med* 1997; 45: 552–560.
27 Harrell FE. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York, Springer, 2001.
28 Park M, Hastie T. L1-regularization path algorithm for generalized linear models. *J R Statist Soc B* 2007; 69: 659–677.
29 Maisel AS, Krishnaswamy P, Nowak RM, *et al.* Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. *N Engl J Med* 2002; 347: 161–167.
30 Andersson B, Hall C. N-terminal proatrial natriuretic peptide and prognosis in patients with heart failure and preserved systolic function. *J Card Fail* 2000; 6: 208–213.
31 Pfisterer M, Buser P, Rickli H, *et al.* BNP-guided *vs* symptom-guided heart failure therapy: the Trial of Intensified *vs* Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. *JAMA* 2009; 301: 383–392.
32 Levitt JE, Vinayak AG, Gehlbach BK, *et al.* Diagnostic utility of B-type natriuretic peptide in critically ill patients with pulmonary edema: a prospective cohort study. *Crit Care* 2008; 12: R3.
33 Meyer B, Huelsmann M, Wexberg P, *et al.* N-terminal pro-B-type natriuretic peptide is an independent predictor of outcome in an unselected cohort of critically ill patients. *Crit Care Med* 2007; 35: 2268–2273.
34 Morgenthaler NG, Struck J, Christ-Crain M, *et al.* Pro-atrial natriuretic peptide is a prognostic marker in sepsis, similar to the APACHE II score: an observational study. *Crit Care* 2005; 9: R37–R45.
35 Berdal JE, Stavem K, Omland T, *et al.* Prognostic merit of N-terminal-proBNP and N-terminal-proANP in mechanically ventilated critically ill patients. *Acta Anaesthesiol Scand* 2008; 52: 1265–1272.
36 Leuchte HH, Holzapfel M, Baumgartner RA, *et al.* Clinical significance of brain natriuretic peptide in primary pulmonary hypertension. *J Am Coll Cardiol* 2004; 43: 764–770.
37 Yap LB, Mukerjee D, Timms PM, *et al.* Natriuretic peptides, respiratory disease, and the right heart. *Chest* 2004; 126: 1330–1336.
38 Stolz D, Breidthardt T, Christ-Crain M, *et al.* Use of B-type natriuretic peptide in the risk stratification of acute exacerbations of COPD. *Chest* 2008; 133: 1088–1094.
39 Villar J, Blazquez MA, Lubillo S, *et al.* Pulmonary hypertension in acute respiratory failure. *Crit Care Med* 1989; 17: 523–526.
40 Vickery S, Price CP, John RI, *et al.* B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with CKD: relationship to renal function and left ventricular hypertrophy. *Am J Kidney Dis* 2005; 46: 610–620.
41 Mohapatra SS. Role of natriuretic peptide signaling in modulating asthma and inflammation. *Can J Physiol Pharmacol* 2007; 85: 754–759.
42 Vollmar AM. The role of atrial natriuretic peptide in the immune system. *Peptides* 2005; 26: 1086–1094.
43 Aiura K, Ueda M, Endo M, *et al.* Circulating concentrations and physiologic role of atrial natriuretic peptide during endotoxic shock in the rat. *Crit Care Med* 1995; 23: 1898–1906.
44 Vaz Perez A, Doehner W, von Haehling S, *et al.* The relationship between tumor necrosis factor-α, brain natriuretic peptide and atrial natriuretic peptide in patients with chronic heart failure. *Int J Cardiol* 2010; 141: 39–43.
45 Lisboa T, Diaz E, Sa-Borges M, *et al.* The ventilator-associated pneumonia PIRO score: a tool for predicting ICU mortality and health-care resources use in ventilator-associated pneumonia. *Chest* 2008; 134: 1208–1216.
46 Christ-Crain M, Stolz D, Bingisser R, *et al.* Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia: a randomized trial. *Am J Respir Crit Care Med* 2006; 174: 84–93.
47 Stolz D, Christ-Crain M, Bingisser R, *et al.* Antibiotic treatment of exacerbations of COPD: a randomized, controlled trial comparing procalcitonin-guidance with standard therapy. *Chest* 2007; 131: 9–19.
48 Schuetz P, Christ-Crain M, Thomann R, *et al.* Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. *JAMA* 2009; 302: 1059–1066.
49 Bouadma L, Luyt CE, Tubach F, *et al.* Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial. *Lancet* 2010; 375: 463–474.
50 Hillas G, Vassilakopoulos T, Plantza P, *et al.* CRP and PCT as predictors of survival and septic shock in ventilator-associated pneumonia. *Eur Respir J* 2010; 35: 805–811.
51 Bertolini G, D'Amico R, Apolone G, *et al.* Predicting outcome in the intensive care unit using scoring systems: is new better? A comparison of SAPS and SAPS II in a cohort of 1,393 patients. GiViTi Investigators (Gruppo Italiano per la Valutazione degli interventi in Terapia Intensiva). Simplified Acute Physiology Score. *Med Care* 1998; 36: 1371–1382.
52 Leroy O, Meybeck A, d'Escrivan T, *et al.* Impact of adequacy of initial antimicrobial therapy on the prognosis of patients with

ventilator-associated pneumonia. *Intensive Care Med* 2003; 29: 2170–2173.
53 Vincent JL, de Mendonca A, Cantraine F, *et al.* Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on ''sepsis-related problems'' of the European Society of Intensive Care Medicine. *Crit Care Med* 1998; 26: 1793–1800.
54 Ferreira FL, Bota DP, Bross A, *et al.* Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA* 2001; 286: 1754–1758.
55 Vincent JL, Opal SM, Marshall JC. Ten reasons why we should NOT use severity scores as entry criteria for clinical trials or in our treatment decisions. *Crit Care Med*, 38: 283–287.
56 Cohen NH. The real reasons not to rely on severity scores. *Crit Care Med* 2010; 38: 334–335.
57 Murray LS, Teasdale GM, Murray GD, *et al.* Does prediction of outcome alter patient management? *Lancet* 1993; 341: 1487–1491.
58 Marrie TJ, Lau CY, Wheeler SL, *et al.* A controlled trial of a critical pathway for treatment of community-acquired pneumonia. CAPITAL Study Investigators. Community-Acquired Pneumonia Intervention Trial Assessing Levofloxacin. *JAMA* 2000; 283: 749–755.
59 Angus DC, Laterre PF, Helterbrand J, *et al.* The effect of drotrecogin alfa (activated) on long-term survival after severe sepsis. *Crit Care Med* 2004; 32: 2199–2206.
60 Eichacker PQ, Parent C, Kalil A, *et al.* Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. *Am J Respir Crit Care Med* 2002; 166: 1197–1205.
61 Rello J, Rue M, Jubert P, *et al.* Survival in patients with nosocomial pneumonia: impact of the severity of illness and the etiologic agent. *Crit Care Med* 1997; 25: 1862–1867.