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Original Research

CRITICAL CARE

Pancreatic Stone Protein

A Marker of Organ Failure and Outcome in Ventilator-Associated Pneumonia

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Background: **Ventilator-associated pneumonia (VAP) is the most common hospital-acquired, life-threatening infection. Poor outcome and health-care costs of nosocomial pneumonia remain a global burden. Currently, physicians rely on their experience to discriminate patients with good and poor outcome. However, standardized prognostic measures might guide medical decisions in the future. Pancreatic stone protein (PSP)/regenerating protein (reg) is associated with inflammation, infection, and other disease-related stimuli. The prognostic value of PSP/reg among critically ill patients is unknown. The aim of this pilot study was to evaluate PSP/reg in VAP.**

Methods: **One hundred one patients with clinically diagnosed VAP were assessed. PSP/reg was retrospectively analyzed using deep-frozen serum samples from VAP onset up to day 7. The main end point was death within 28 days after VAP onset.**

Results: **Serum PSP/reg was associated with the sequential organ failure assessment score from VAP onset (Spearman rank correlation coefficient 0.49 $P < .001$) up to day 7. PSP/reg levels at VAP onset were elevated in nonsurvivors (n = 20) as compared with survivors (117.0 ng/mL [36.1-295.3] vs 36.3 ng/mL [21.0-124.0] $P = .011$). The areas under the receiver operating characteristic curves of PSP/reg to predict mortality/survival were 0.69 at VAP onset and 0.76 at day 7. Two PSP/reg cutoffs potentially allow for identification of individuals with a particularly good and poor outcome. Whereas PSP/reg levels below 24 ng/mL at VAP onset were associated with a good chance of survival, levels above 177 ng/mL at day 7 were present in patients with a very poor outcome.**

Conclusions: **Serum PSP/reg is a biomarker related to organ failure and outcome in patients with VAP.**

Trial registry: **ISRCTN.org; No.: ISRCTN61015974; URL: www.isrctn.org.**

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Abbreviations: AUC = area under the curve; CPIS = clinical pulmonary infection score; IQR = interquartile range; MAP = mean arterial pressure; ODIN = organ dysfunction and/or infection; PSP = pancreatic stone protein; reg = regenerating protein; ROC = receiver operating characteristic; SAPS = simplified acute physiologic score; SOFA = sequential organ failure assessment; VAP = ventilator-associated pneumonia

Ventilator-associated pneumonia (VAP) is a major complication of critically ill patients. Despite improved preventive measures, a substantial number of intubated patients still develop VAP. VAP profoundly contributes to morbidity and health-care costs.[1] The increasing importance and high prevalence of drug-resistant pathogens make VAP an important field of research. While attributable mortality of VAP is unknown, crude mortality remains high.[2-4] Predictive tools which allow for objective assessment of risk in patients with VAP are limited.[5] Risk stratification is the mainstay of management in many diseases, nevertheless it is rather new in critical care. Potential applications of risk stratification in intensive care include patient information and surrogate markers in clinical trials.[6] Furthermore, risk-adapted therapy might improve intensive care in the future.

Pancreatic stone protein (PSP), regenerating protein (reg), and lithostathine are different names for

an identical 16-kDa polypeptide belonging to the family of lectin-binding proteins.[7] Trypsin cleavage at a particular site allows polymerization into fibrils, resistant to further cleavage. Several PSP/reg-releasing stimuli, signaling pathways, and functions have been reported, mainly in regard to the pancreas.[8,9] The first function suggested was the capacity to inhibit pancreatic stone formation, a role subsequently challenged.[10,11] Independently, PSP/reg appeared to stimulate islet β-cell growth and regeneration.[7,10] In recent years, PSP/reg investigations expanded beyond the pancreas. PSP/reg elevations were reported in conditions of inflammation and infection, and due to surgical stimuli.[12] Preliminary data show that PSP/reg might be superior to procalcitonin in discriminating patients with infection, infection with sepsis, and no infection.[13] Its immediate upregulation in the critically ill warrants further investigation.

In this pilot study investigating patients with VAP, we assessed whether serum PSP/reg is associated with organ failure and survival. To our knowledge, this is the first trial investigating PSP/reg at medical and surgical ICUs.

Materials and Methods

Setting and Study Population

Data and serum samples were collected within a multicentric trial, including 101 patients with clinically diagnosed VAP.[14,15] PSP/reg was analyzed retrospectively. In brief, the main objective of the trial was to evaluate procalcitonin-guided antibiotic de-escalation in VAP as compared with usual care. The study took place at seven medical and surgical ICUs (UMass Memorial Medical Center, Worcester, Massachusetts; University Hospital Lausanne, Lausanne, Switzerland; and University Hospital Basel, Basel, Switzerland) from August 2006 to December 2007. The study was approved by the institutional review boards of all participating institutions. 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.[16] 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°C/100.4°F), leukocytosis/-penia (leukocyte count > 11,000/μL or < 3000/μL). VAP patients were eligible for the study if they were intubated for mechanical ventilation for at least 48 h and older than 18 years. Patients were excluded if they were pregnant, were enrolled in another trial, had received immunosuppressants or long-term corticosteroid therapy (above 0.5 mg/kg per day for longer than 1 month), were immunosuppressed, or had a coexisting extrapulmonary infection diagnosed in the first 3 days requiring antibiotic therapy for more than 3 days.

Baseline Assessment and Follow-up

At time of enrollment, the following information was recorded from each subject: age, sex, preexisting comorbidities, 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, Pao_2/Fio_2, leukocyte count (WBC), and PSP/reg serum levels. The following indices were calculated: simplified acute physiologic score (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, Pao_2/Fio_2, WBC, SOFA, ODIN, and CPIS, and mechanical ventilation status and antibiotic use and survival throughout the 28-day study period. Serum PSP/reg levels were determined on VAP onset (day 0) and for 6 consecutive days after VAP diagnosis (days 2-7).

Outcome Assessment

Follow-up was for 28 days or until death. Patients who died within 28 days after VAP onset were classified as nonsurvivors, all others were classified as survivors. No patient was lost to follow-up.

PSP/reg Measurement

Deep-frozen serum samples were used for PSP/reg measurements. PSP/reg was analyzed using an isoform-specific enzyme-linked immunosorbent assay as described previously.[13,17] In brief, serum samples were incubated with guinea pig anti-PSP/reg antibody precoated plates. Rabbit anti-PSP/reg was added and subsequently detected by phosphatase-conjugated anti-rabbit IgG. A detection limit of under 0.1 ng/mL and an interplate variance of under 10% was reported previously.[13]

Statistical Analyses

Discrete variables are expressed as counts (percentages) and continuous variables as median (interquartile range [IQR]). Comparability of groups was analyzed by the χ^2 test, the Fisher exact test, or the Mann-Whitney *U* test, as appropriate. Correlation

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analyses were performed using the Spearman rank correlation coefficient. To detect the time course of the biomarkers across survivors and nonsurvivors, a linear mixed-effect model with fixed factors day, group, and random factor subject was performed on log-transformed parameters. To study possible different time courses, the interaction between day and group was determined. Values of the markers at VAP onset were also included to adjust for potential different baseline values in the study groups. Δ PSP/reg was calculated by subtracting PSP/reg levels of day 2 or 4 from levels of VAP onset. Logistic regression was used to analyze the predictive value of PSP/reg and ε PSP/reg. Logistic regression models were calculated on log-transformed parameters. The OR represents the change in odds in being in one category of outcome (survivors vs nonsurvivors) when the value of predictor (lnPSP/reg) increases by one unit. Time to death was analyzed by Kaplan-Meier survival curves and compared by the log-rank test. Area-under-the-curve (AUC) values of receiver operating characteristic (ROC) curves were calculated. AUC values were reported with corresponding 95% CI. Cutoffs were identified using the Youden index (maximal difference between sensitivity and 1-specificity). Likelihood ratios were calculated, using sensitivity and specificity, to evaluate test performance (a specificity of maximal 99% was used). All tests were two tailed; $P < .05$ was defined as significant. Data were analyzed using statistical software (Statistical Package for Social Sciences, Version 16 for Windows; SPSS; Chicago, Illinois).

Results

One hundred one patients with a median [IQR] age of 57 years [43-70] participated in the study. Detailed baseline characteristics for survivors and nonsurvivors are summarized in Table 1. Despite high antibiotic pretreatment within 14 days prior to study inclusion (75%), respiratory specimens identified a causative organism in 74 patients (76%). The most frequent pathogens were *Staphylococcus aureus* (30%), *Pseudomonas aeruginosa* (25%), and *Klebsiella* species (13%). 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 (20%) 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). In seven patients, PSP/reg values at VAP onset were missing. There was no patient with an undetectable PSP/reg level. No patient was lost to follow-up.

Median [IQR] PSP/reg at VAP onset (day 0) was 38.6 ng/mL [22.2-179.0] and weakly correlated with age and MAP (Spearman rank correlation coefficient 0.23 $P = .027$ and -0.23 $P = .026$, respectively). There was no association of PSP/reg day 0 with sex, comorbidities (renal, pulmonary, cardiac, hematologic/oncologic) and gas exchange (oxygen saturation, PaO_2/FIO_2). Furthermore, PSP/reg levels during the first 7 days did not correlate with the duration of antibiotic therapy (day 0-7, $P \geq .05$). We found a significant correlation between PSP/reg day 0 and SOFA day 0 (Spearman rank correlation coefficient, 0.49; $P < .001$; Fig 1). A moderate correlation between daily PSP/reg and SOFA was present up to 7 days after VAP onset (days 2-7, Spearman rank correlation coefficient > 0.3; $P < .05$). PSP/reg day 0 levels were significantly elevated in nonsurvivors as compared with survivors (117.0 ng/mL [36.1-295.3] vs 36.3 ng/mL [21.0-124.0]; $P = .011$). There was no interaction between day and group, reflecting a similar decrease of PSP/reg, in survivors and nonsurvivors ($P = .84$; Fig 2). PSP/reg kinetics within 2 or 4 days after VAP onset (ε PSP/reg) did not indicate outcome ($P = .62$ and 0.72, respectively). PSP/reg day 0 tertiles differed significantly in survival (log rank; $P = .014$; Fig 3). In univariate logistic regression, PSP/reg day 0 and day 7 were significant predictors of survival (day 0, OR 1.60, 95% CI, 1.07-2.38, $P = .022$; day 7, OR 2.36, 95% CI, 1.27-4.39, $P = .007$). In ROC analysis, the AUC of PSP/reg for mortality/survival on VAP onset and after 7 days was 0.69 and 0.76 (95% CI, 0.57-0.80 and 0.62-0.91), respectively. We identified a PSP/reg cutoff of 24 ng/mL at VAP onset as the most accurate threshold for predicting survival (Fig 4). The sensitivity was 36% and specificity 100% for predicting survival (likelihood ratio positive, 36; likelihood ratio negative, 0.65). In contrast, a PSP/reg threshold of 177 ng/mL at day 7 after VAP onset was the most valuable parameter to predict death (Fig 4B). Sensitivity and specificity were 58% and 91% (likelihood ratio positive, 6.4; likelihood ratio negative, 0.46). Positive and negative predictive values were 54% and 90%, accordingly. The OR for patients PSP/reg above 177 ng/mL at day 7 to die until day 28 was 13.8 (95% CI, 3.3-57.1).

Discussion

In this pilot study investigating serum PSP/reg in VAP, we report three new findings. First, nonsurviving VAP patients have significantly elevated PSP/reg levels. Second, PSP/reg reflects organ dysfunction/failure. Third, PSP/reg discriminates survivors from nonsurvivors and potentially stratifies patients with good and poor outcome.

To our knowledge, PSP/reg levels were neither investigated in VAP nor as a measure of outcome. Herein, we report that PSP/reg levels at VAP onset are elevated in patients who die within subsequent 28 days. Importantly, age, sex, duration of antibiotic therapy, and specific comorbidities (such as hematologic/oncologic) irrespective of disease severity seem not to influence PSP/reg levels. It is noteworthy that the exact origin of PSP/reg in critically ill patients is

Table 1—*Demographics of 101 Patients With VAP at Study Inclusion*

Characteristics	Total (N = 101)	Survivors (n = 81)	Nonsurvivors (n = 20)	*P* Value
Age, y	57 [43-70]	55 [42-68]	67 [52-75]	.033
Male sex	74 (74)	61 (76)	13 (65)	.459
Admission				
Medical	53 (53)	41 (51)	12 (60)	.615
Elective surgery	4 (4)	4 (5)	0 (0)	.582
Emergency surgery	43 (43)	35 (43)	8 (40)	.994
From home	61 (62)	51 (65)	10 (47)	.291
From hospital	20 (20)	15 (19)	5 (24)	.517
From other ICU	18 (18)	12 (15)	6 (29)	.114
Coexisting illnesses				
Cardiac	48 (48)	37 (46)	11 (55)	.619
Pulmonary	19 (19)	14 (17)	5 (25)	.523
Renal	16 (16)	11 (14)	5 (25)	.302
Hematologic/oncologic	8 (8)	4 (5)	4 (20)	.047
Reason for mechanical ventilation				
Cardiovascular failure	32 (32)	25 (31)	7 (35)	.930
Acute respiratory failure	53 (53)	42 (52)	11 (55)	.998
Trauma	33 (33)	28 (35)	5 (25)	.582
Neurologic failure	30 (30)	26 (32)	4 (20)	.431
Sepsis	25 (25)	22 (27)	3 (15)	.387
Miscellaneous	10 (10)	9 (11)	1 (5)	.682
Duration of mechanical ventilation before VAP, d	6 [3.5-9]	6 [3-9]	5.5 [4-9.8]	.801
Antibiotics within 14 d before VAP onset	76 (75)	59 (73)	17 (85)	.387
Vital parameters at VAP onset				
Heart rate/min	90 [79-103]	90 [80-110]	88 [76-95]	.344
MAP, mmHg	77 [70-90]	77 [70-90]	75 [68-85]	.436
Temperature, °C	38.0 [37.4-38.6]	38.1 [37.6-38.8]	37.4 [37.0-38.3]	.010
PaO_2/FIO_2	188 [135-253]	192 [140-254]	173 [126-225]	.290
SaO_2, %	97 [94-99]	97 [94-99]	98 [96-99]	.468
WBC, 10^9/L	11.6 [8.0-15.0]	11.2 [8.0-14.6]	13.3 [9.3-18.0]	.209
Microbiology				
Positive microbiological cultures (EA, BAL, PSB)	74 (76)	58 (73)	16 (89)	.226
Positive blood cultures	34 (34)	29 (36)	5 (25)	.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]	.002
ODIN score	2 [1-3]	2 [1-2]	3 [1-4]	.050
SOFA score	7.0 [6.0-9.8]	6 [5-9]	9 [7-14]	.002
CPIS	7.5 [6.0-9.0]	8.0 [6.0-9.0]	7.0 [6.0-8.0]	.799

Discrete variables are expressed as counts (%) and continuous variables as median [interquartile range]. CPIS = clinical pulmonary infection score; EA = endotracheal aspirate; MAP = mean arterial pressure; ODIN = organ dysfunction and/or infection; PSB = protected-specimen brush; SaO_2 = arterial oxygen saturation; SAPS = simplified acute physiologic score; SOFA = sequential related organ failure assessment; VAP = ventilator-associated pneumonia.

not known. Pancreatic acinar cells are considered as the most important source of PSP/reg under normal conditions, whereas other tissues contribute to PSP/reg levels mainly in pathologic conditions.[9,18-21] At first, PSP/reg was proposed as a marker of pancreatic injury. Subsequent investigations reported that PSP/reg levels did not increase even after the most severe pancreatic injury and therefore challenged this issue.[22] Moreover, several data demonstrate PSP/reg elevations unrelated to pancreatic disease.[21,23,24] In our population, pancreatitis was present only in a single patient. Interestingly, this patient had a PSP/reg of 22.8 ng/mL at VAP onset and a maximum PSP/reg of 27.0 ng/mL. Both PSP/reg levels were below the median of survivors, to which group the patient belonged. Therefore, other releasing mechanisms and origins probably dominate.

One of the most important PSP/reg studies performed so far investigated infectious complications of polytraumatic patients without pancreatic injury.[13] Early during hospitalization, PSP/reg was elevated in all trauma patients as compared with healthy control subjects. After the fifth day of trauma, PSP/reg levels were continuously increased in patients with infection as compared with trauma patients who did not develop an infectious complication. Differences between the infection and infection plus sepsis group were present on most days. The study indicates that PSP/reg might mirror inflammatory and/or infectious conditions. This is not surprising since proinflammatory

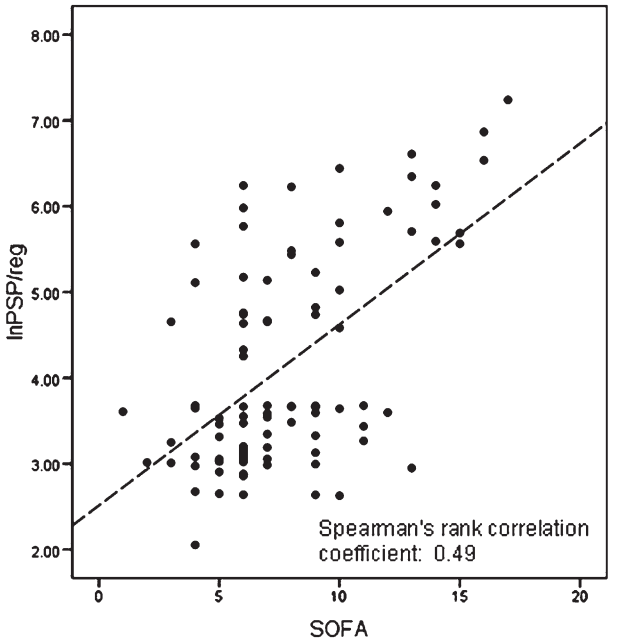

FIGURE 1. Scatter plot demonstrating the correlation of SOFA and lnPSP/reg at onset of ventilator-associated pneumonia (VAP) ($P < .001$). PSP/reg was logarithmized for graphical demonstration. Regression line (dashed line). PSP/reg = pancreatic stone protein/regenerating protein; SOFA = sequential related organ failure assessment.

cytokines such as tumor necrosis factor α, interferon γ, and IL-6 are known to induce PSP/reg expression.[25] Thus, PSP/reg can be assumed as a potential sepsis marker. A role in sepsis is further encouraged by certain functional properties of PSP/reg. It was shown that PSP/reg itself, as well as the cleaved form, aggregate bacteria.[26] Moreover, in neutrophil granulocytes, PSP/reg induced a shedding of L-selectin and a downregulation of β_2-integrin indicating a neutrophil activation.[13] Briefly, the knowledge of PSP/reg in inflammatory conditions is scarce. Nonetheless, it is likely to participate in the inflammatory response.

Another important observation is that pancreatic upregulation of PSP/reg occurs due to several stimuli such as intraperitoneal saline infusion and surgery.[9,27-29] Interestingly, we could show a fairly good correlation of PSP/reg with SOFA at VAP onset. SOFA is a frequently used measure of organ function and organ failure in intensive care. Consequently, we believe that deteriorations of distinct cells and organs cause PSP/reg elevations. PSP/reg levels at VAP onset (median, 38.6 ng/mL) were clearly higher than those previously reported in healthy subjects (median, 10.4 ng/mL; IQR [7.5-12.3]).[13] Hence, one or several stimuli increasing PSP/reg levels have to be evident in VAP patients. Those releasing factors might be further aggravated in patients with more severe disease and worse outcome. Nevertheless, there probably exists a small PSP/reg overlap in VAP patients and healthy individuals. However, those VAP patients are considered to have a good outcome.

Besides different PSP/reg levels among survivors and nonsurvivors, we report predictive properties of PSP/reg regarding survival. Several methodological approches, including logistic regression, Kaplan-Meier, and ROC-curve analysis, identified PSP/reg as a significant predictor of outcome. Moreover, when hospital mortality was used rather than 28-day mortality, similar results were obtained (data not shown). Of note, the exact predictive value is not known and a PSP/reg increase (ε PSP/reg) during ICU stay did not indicate worse outcome. When PSP/reg and SOFA were combined, predictive values were slightly better than every single parameter (data not shown). However, this improvement did not reach statistical significance. Nonetheless, the combination of PSP/reg and clinical scores might prove useful in the future. Performance, clinical impact, and, particularly, the cost of predictive tools need to be addressed in future trials.

For future risk assessment, we propose two cutoff values for predicting survival and death. Both cutoffs are characterized by a high specificity. A PSP/reg cutoff of 24 ng/mL at VAP onset had the highest accuracy to identify survivors. Patients below this value had a particularly good outcome independent of age and comorbidities. Conversely, we suggest a PSP/reg threshold of 177 ng/mL at day 7 to determine patients with a poor chance of survival. These cutoffs have a high likelihood ratio positive and small likelihood ratio negative. Thus, negative results (day 0, > 24 ng/mL; day 7, < 177 ng/mL) need to be interpreted cautiously. Risk assessment at day 7 is considered as a follow-up. It is noteworthy that patients deceased or

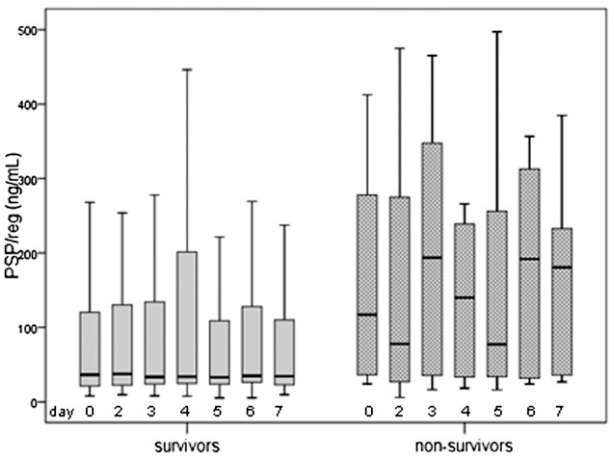

FIGURE 2. Overview of PSP/reg levels during the first 7 days after VAP onset in survivors and nonsurvivors. Boxes represent the interquartile range; whiskers include 1.5 times the interquartile range. For clear graphical presentation, outliers are not displayed. See Figure 1 legend for expansion of abbreviations.

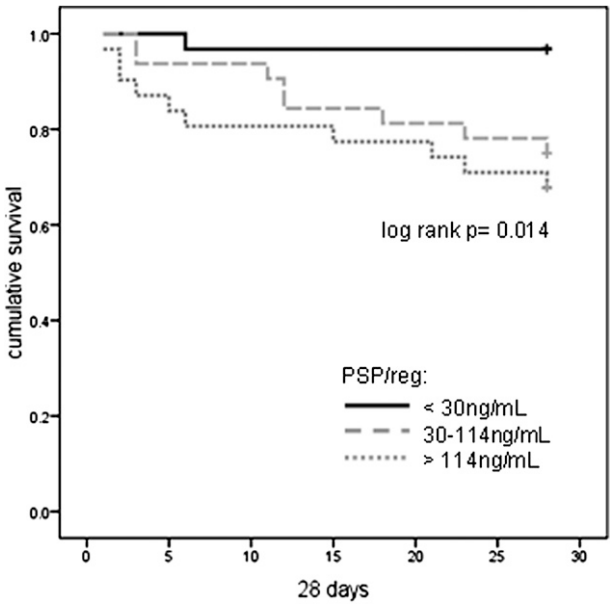

FIGURE 3. Kaplan-Meier estimates of survival within 28 days of VAP onset. Ninety-four patients were stratified into PSP/reg tertiles at VAP onset. See Figure 1 legend for expansion of abbreviations.

discharged early are missed, and pneumonia is resolved in most patients at day 7. However, with this approach, approximately 50% of our population were stratified either to the low-risk or to the high-risk group. The remaining 50% were discharged before day 7 or supposed to be at intermediate risk.

Importantly, we have to point out a few limitations. First, the novel study findings need to be considered hypothesis generating. Hence, the exact performance of PSP/reg in predicting survival remains unclear. Importantly, the costs of a biomarker have to be taken into account and balanced against its contribution to risk determination. Although the study was performed in several centers, external validation of our results is essential. Therefore, PSP/reg measurements cannot yet be recommended. Second, the study size did not allow the evaluation of subgroups and multivariate analysis. For that reason, an adjustment for age and comorbidities was not feasible. This also applies for pancreatic injury. Despite the low rate of pancreatitis, we cannot rule out subclinical pancreatic injury which was present in more patients and influenced the results. Furthermore, an effect of antibiotic duration or number of antibiotics used cannot be ruled out. Therefore, future trials need to analyze potential confounders in regard to PSP/reg predicting survival. Third, clinical diagnosis of VAP has a limited specificity. Thus, non-VAP patients might have biased our results either in a positive or negative way. However, in line with previous authors, we believe that the clinical approach recommended by the American Thoracic Society is the most accurate

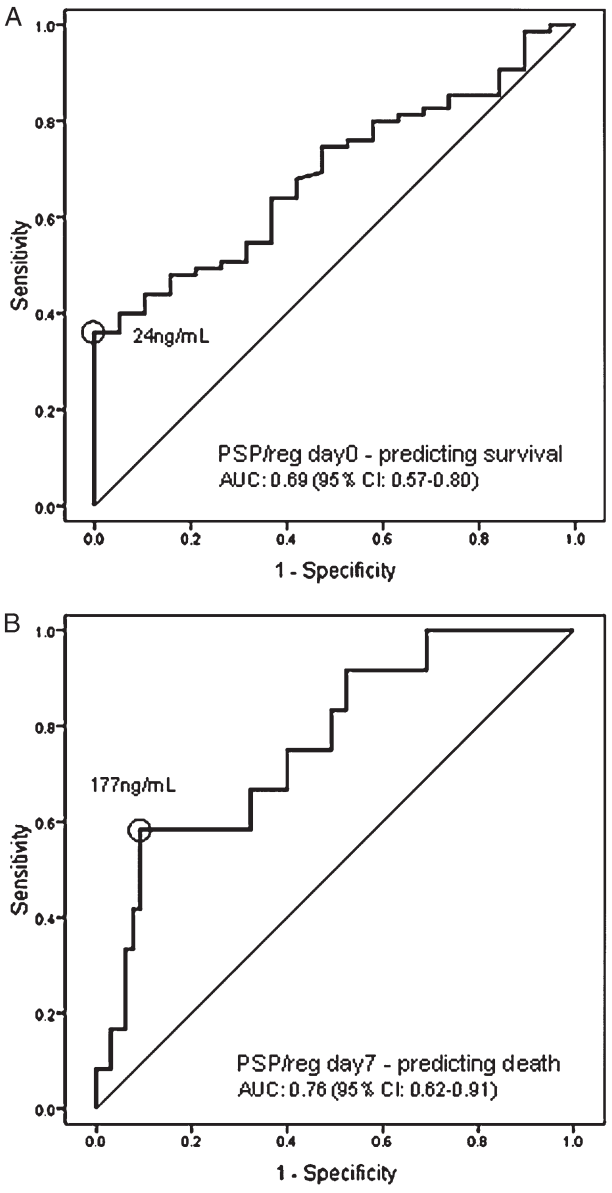

FIGURE 4. A-B, PSP/reg receiver operating characteristic curves: day 0 to predict survival (A), day 7 to predict death (B). Circles highlight the most accurate cutoffs. A, Using the 24 ng/mL cutoff at VAP onset reveals a sensitivity of 36% and a specificity of 100% to predict survival. B, The best threshold to predict death (sensitivity: 54%, specificity: 90%) was 177 ng/mL at day 7. AUC = area under the curve. See Figure 1 legend for expansion of other abbreviations.

for starting VAP-specific treatment.[30] Furthermore, PSP/reg performance may not be limited to VAP patients. Hence, forthcoming trials have to address PSP/reg in other populations.

We conclude that PSP/reg is a biomarker reflecting organ failure and outcome in VAP. Moreover, PSP/reg has discriminative properties and may allow for identification of survivors and nonsurvivors. Future trials are required to reassess performance and evaluate the impact of PSP/reg measurements in intensive care.

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Dr Boeck: contributed to study conception and design, data analysis and interpretation, and drafting the manuscript for important intellectual content.
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Dr Stolz: contributed to study conception and design, data analysis and interpretation, and drafting the manuscript for important intellectual content.
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