CHEST

Original Research

COPD

Pancreatic Stone Protein Predicts Positive Sputum Bacteriology in Exacerbations of COPD

Andreas Scherr, MD; Rolf Graf, MD; Martha Bain, RN; Mirjam Christ-Crain, MD; Beat Müller, MD; Michael Tamm, MD, FCCP; and Daiana Stolz, MD, MPH

Background: **Pancreatic stone protein/regenerating protein (PSP/reg) serum levels are supposed to be increased in bacterial inflammation. PSP/reg levels also might be useful, therefore, as a predictor of bacterial infection in COPD.**
Methods: **Two hundred consecutive patients presenting to the ED due to acute exacerbation of COPD were prospectively assessed. Patients were evaluated based on clinical, laboratory, and lung functional parameters at admission (exacerbation) and after short-term follow-up (14-21 days). PSP/reg serum values were measured by a newly developed enzyme-linked immunosorbent assay.**
Results: **PSP/reg levels were elevated in subjects with COPD exacerbation (23.8 ng/mL; 95% CI, 17.1-32.7) when compared with those with stable disease (19.1 ng/mL; 95% CI, 14.1-30.4; $P = .03$) and healthy control subjects (14.0 ng/mL; 95% CI, 12.0-19.0; $P < .01$). Higher PSP/reg values were observed in exacerbations with positive sputum bacteriology compared with those with negative sputum bacteriology (26.1 ng/mL [95% CI, 19.2-38.1] vs 20.8 ng/mL [95% CI, 15.6-27.2]; $P < .01$). Multivariate regression analysis revealed PSP/reg level as an independent predictor of positive sputum bacteriology. A combination of a PSP/reg cutoff value of > 33.9 ng/mL and presence of discolored sputum had a specificity of 97% to identify patients with pathogenic bacteria on sputum culture. In contrast, PSP/reg levels < 18.4 ng/mL and nonpurulent sputum ruled out positive bacterial sputum culture (sensitivity, 92%). In survival analysis, high PSP/reg levels at hospital admission were associated with increased 2-year mortality.**
Conclusions: **Serum PSP/reg level might represent a promising new biomarker to identify bacterial etiology of COPD exacerbation.**
Trial registry: **Current Controlled Trials Database; No.: ISRCTN-77261143; URL: http://www.controlled-trials.com/isrctn**
CHEST 2013; 143(2):379–387

Abbreviations: AECOPD = acute exacerbation in COPD; CRP = C-reactive protein; GOLD = Global Initiative for Chronic Obstructive Lung Disease; PCT = procalcitonin; PSP/reg = pancreatic stone protein

Severe exacerbations are the major cost drivers in COPD and are frequently associated with impaired health-related quality of life.[1,2] Infection is usually considered the main cause of acute exacerbation in COPD (AECOPD).[3,4] The exact role of bacteria in AECOPD and the usefulness of antibiotics in this setting are unclear.[5-9] Whereas patients with clinical signs (eg, sputum purulence) of bacterial infection seem to benefit from antibiotics, unselected prescription of antibiotics is ineffective.[10-12] Specific biomarkers with predictive characteristics could assist in selecting patients benefiting the most from antibiotic treatment. Accordingly, procalcitonin (PCT) guidance offered a sustained advantage over standard therapy in reducing antibiotic use in COPD.[13] However, PCT was neither associated with sputum purulence nor positive sputum cultures.[14]

Pancreatic stone protein/regenerating protein (PSP/reg) is a 16-kDA polypeptide belonging to the family of lectin-binding proteins.[15] Several PSP/reg-releasing stimuli have been reported, mainly in regard to the pancreas.[16,17] Current investigations suggest that elevated PSP/reg levels might also be associated with bacterial infection.[18] PSP/reg levels were also considered to reflect organ failure and outcome in ventilator-associated pneumonia.[19] Preliminary data showed that PSP/reg level might be superior to PCT guidance in differentiating between local infection, sepsis, and

absence of infection.[20] We investigated whether circulating PSP/reg levels are associated with bacterial growth on sputum culture and outcome in AECOPD.

Materials and Methods

Setting and Study Population

The trial was designed as an investigator-initiated and investigator-driven, prospective, monocentric study. Data were analyzed from 200 patients included in a randomized trial and admitted from November 2003 to March 2005 for AECOPD to the ED of the University Hospital of Basel, Switzerland. A complete description has been reported elsewhere.[13] The trial was approved by the Basel Ethics Committee (approval number 232/03) and was registered with the Current Controlled Trials Database (Procalcitonin-Guided Antibiotic Therapy in Acute Exacerbations of Chronic Obstructive Pulmonary Disease [COPD] [AECOPD]: a Randomised Trial-The ProCOLD Study; ISRCTN77261143). All participants gave written informed consent.

In brief, the primary end point of the study was to evaluate prescription and duration of antibiotic use in patients randomly assigned to PCT guidance compared with usual care. To be eligible for the study, AECOPD had to be diagnosed on the basis of clinical history, physical examination, chest radiograph, and post-bronchodilator spirometric criteria for COPD according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) guidelines within 48 hours after admission.[21] At recovery (days 14-21), diagnosis of COPD was reevaluated by lung function testing. Patients were excluded from participation in cases of severe immunosuppression, concomitant pulmonary diseases (eg, bronchial asthma, cystic fibrosis, bronchiectasis), or infiltrates present on actual chest radiograph.

Baseline assessment comprised epidemiologic (eg, sex, age) and clinical data, including medical history (ie, smoking history; duration of COPD; previous AECOPD; prehospitalizations; concomitant medication, including long-term oxygen therapy; and comorbidities). Characteristics of the actual exacerbation episode were documented (ie, vital signs, respiratory symptoms, and routine blood chemistry, including leukocyte counts and arterial blood gas analysis). Blood samples for biomarker measurement were obtained. Spontaneously expectorated sputum samples were collected and immediately transported to the laboratory. For examination, standard techniques as described by the American Society for Microbiology were used.[22] Samples with $< 10^5$/mL epithelial cells and > 25 polymorphonuclear cells per low-power field on Gram-stain results were considered representative. Semiquantitative culture was performed of adequate samples. Positive cultures were defined by presence of pathogenic microorganisms in pure culture or pathogenic organisms in excess (≥ 1 log) to normal flora.

Outcome Measurements

Levels of circulating inflammatory biomarkers (ie, PSP/reg, PCT, and C-reactive protein [CRP]) were measured at admission, before institution of medical therapy, and at recovery (days 14-21). At follow-up (after 6 and 24 months) re-exacerbations, extent and timing of health-care use were retrospectively assessed and, if necessary, confirmed by medical records and family physicians. Sputum specimens were used to assess correlations of PSP/reg level, discolored sputum, and microbiologic analysis. Secondary end points of the ProCOLD trial were duration of hospital stay, need for ICU admission, in-hospital mortality, time to re-exacerbation, and 2-year mortality.[13] For survival analysis, patients were classified as survivors or nonsurvivors at 2-year follow-up.

Determination of PSP/reg Serum Concentration

At admission and at recovery (days 14-21), whole blood was collected from subjects into sterile tubes containing 143 International Units lithium heparin. Probes were allowed to clot at 4°C for 15 min before being centrifuged at 1,500*g* for 20 min. The serum was aliquoted and stored at −70°C until the assay was performed. Serum concentrations of PSP/reg were measured using 50 μL serum by a new, not commercially available, sandwich isoform-specific, enzyme-linked immunosorbent assay.[20]

The median physiologic value of serum PSP/reg in healthy control subjects was 10.4 ng/mL (interquartile range, 7.5-12.3 ng/mL).[20] The lower detection limit (analytical sensitivity) of the assay was < 0.1 ng/mL and the interplate variance was $< 10\%$.

Circulating levels of PSP/reg were also analyzed in a control group consisting of 40 elderly, healthy blood donors (mean [SD] age, 59.2 years [± 7 years]; 55% men) and in 133 patients with stable COPD from clinical routine (mean age [SD], 64.9 years [± 12.3 years]; 74% men; mean [SD] $FEV_{1\%}$ predicted, 47% [± 17%]).

Statistical Analysis

Discrete variables are expressed as counts (percentages) and continuous variables as mean (SD) or medians (interquartile range). PSP/reg, CRP, and PCT values were carried out on $\log_{10}$-transformed data to reach normal distribution for parametric analysis. Comparisons of PSP/reg level with clinical variables (eg, GOLD class, Anthonisen criteria, sputum bacteriology) were analyzed by Kruskal-Wallis analysis of variance test, Wilcoxon matched-pair test, or Mann-Whitney *U* test. Correlations of PSP/reg level and clinical variables were calculated using the Spearman correlation. Logistic regression analysis was performed to identity predictor variables for positive sputum cultures. Diagnostic performances of PSP/reg level and sputum color to predict sputum bacteriology were evaluated by receiver operating characteristic analysis. Standard definitions for sensitivity, specificity, and positive and negative predictive values were used. Cutoff points were selected by meaningful differences representing the first and third quartile of PSP/reg levels (18.4 ng/mL and 33.9 ng/mL, respectively). Cumulative risk of death was analyzed by Kaplan-Meier survival curves and compared by the log-rank test. All tests were two tailed; $P < .05$ was defined as significant; data were analyzed using the Statistical Package for Social Sciences, version 19 (IBM).

Manuscript received March 19, 2012; revision accepted July 6, 2012.

Affiliations: From the Clinic of Pulmonary Medicine and Respiratory Cell Research (Drs Scherr, Tamm, and Stolz), and Clinic of Endocrinology (Dr Christ-Crain), Diabetes and Clinical Nutrition, University Hospital, Basel; Pancreatitis Research Laboratory (Dr Graf and Ms Bain), University Hospital Zurich, Zurich; and the Medical University Clinic (Dr Müller), Kantonsspital Aarau AG, Aarau, Switzerland.

Funding/Support: Dr Stolz was supported by grants from the Swiss National Foundation [No. PP00-P3_128412/1]. Additional funding was granted by the Clinic of Pulmonary Medicine and Clinic of Endocrinology, Diabetes and Clinical Nutrition, University Hospital Basel, Basel, Switzerland. Dr Christ-Crain was supported by grants from the Swiss National Foundation [No. PP00P3_123346].

Correspondence to: Daiana Stolz, MD, MPH, Clinic of Pulmonary Medicine and Respiratory Cell Research, University Hospital Basel, Petersgraben 4, CH-4031 Basel, Switzerland; e-mail: dstolz@uhbs.ch

DOI: 10.1378/chest.12-0730

Results

Study Population

Baseline characteristics are illustrated in Table 1 for the whole study population (N = 200) and the subgroup, with available sputum specimens (n = 103) as a function of positive (potentially pathogenic flora) (n = 65) or negative (mouth flora negative for potentially pathogenic flora) (n = 43) culture results. More severe airway obstruction, as suggested by GOLD guidelines,[21] was diagnosed in 133 patients (67%). At least one episode of AECOPD within the previous year was reported by 154 patients (77%). Fifty-one percent of all cases (n = 102) required hospitalization. Fifty percent of patients fulfilled the criteria of type 1 exacerbation, according to the Anthonisen criteria.[5] Ninety-two patients (46%) reported prolonged symptoms of AECOPD (≥4 days) at admission; in 108 patients (64%), symptoms were present for ≤4 days. PSP/reg levels were similar in both groups (20.9 ng/mL [95% CI, 20.5-25.2] vs 23.6 ng/mL [95% CI, 22.4-29.0], $P = .203$).

A total of 43 patients (21.5%) were receiving antibiotics at hospital admission. The mean (SD) duration of previous antibiotic treatment in these cases was 1 day (± 2.8). Sixty-six patients (34%) received oral corticosteroids before hospitalization. PSP/reg levels were similar in patients with and without antibiotics ($P = .105$) and oral corticosteroids ($P = .154$) at hospital admission.

Overall, the mean (SD) length of hospital stay was 11 days (± 21). Admission to the ICU was required for 19 patients (9.5%) and in-hospital mortality was 2% (four patients). At least one re-exacerbation occurred in 85 (42%) of discharged patients. Of these, 38 (45%) required rehospitalization. Twenty-two percent of patients (n = 44) died within 2 years after admission.

A second determination of PSP/reg level was not possible in 13.5% of the cases. PSP/reg levels at initial admission were similar in cases with and without a second determination ($P = .60$).

PSP/reg in Acute Exacerbation, Stable COPD, and at Short-term Follow-up

PSP/reg levels were elevated in AECOPD (23.8 ng/mL; 95% CI, 17.1-32.7) compared with stable COPD (19.1 ng/mL; 95% CI, 14.1-30.4; $P = .03$]) and healthy control subjects (14.0 ng/mL; 95% CI, 12.0-19.0; $P < .01$). PSP/reg level showed weak correlation with Charlson age- and condition-related score ($r = 0.30$, $P < .01$), CRP ($r = 0.15$, $P = .04$), and PCT ($r = 0.34$, $P < .01$). PSP/reg levels were subtly different over categories of Anthonisen classification[5] (type 1, 22.1 ng/mL [95% CI, 17.2-32.94]; type 2, 25.0 ng/mL [95% CI, 18.2-29.7]; type 3, 25.7 ng/mL [95% CI, 18.8-36.8]; $P = .52$).

Independently of antibiotic ($P = .31$) or steroid treatment ($P = .26$), PSP/reg levels remained stable over time (at admission, 24.3 ng/mL [95% CI, 18.4-34.5] vs on recovery, 23.2 ng/mL; [95% CI, 17.9-34.6]; $P = .43$) in the whole study population (n = 200). In contrast, decreasing PSP/reg levels were observed in culture-positive AECOPD following antibiotic treatment (at admission, 30.3 ng/mL [95% CI, 19.5- 41.9] vs on recovery, 22.1 ng/mL [95% CI, 18.3-36.3]; $P = .04$). No decrease in PSP/reg levels was noted in patients receiving antibiotic treatment in culture-negative AECOPD (at admission, 23.1 ng/mL [95% CI, 17.6-29.8] vs recovery, 23.9 ng/mL [95% CI, 19.1-35.6]; $P = .10$).

PSP/reg and Clinical Outcome

PSP/reg levels correlated weakly with length of hospital stay (PSP/reg < 18.4 ng/mL at 6.9 days [SD ± 7.6] vs 11.3 days [SD ± 7.4] for PSP/reg > 33.9 ng/mL, $r = 0.23$, $P < .01$). PSP/reg levels were not associated with the need for ICU admission ($P = .24$), in-hospital mortality ($P = .90$), re-exacerbation ($P = .45$), or rehospitalization after discharge ($P = .65$). Pneumonia developed in 12 patients (6%) during the first 10 days following hospital admission. Microbiologic sputum analyses revealed two positive culture samples each for *Haemophilus influenzae* and *Streptococcus pneumoniae*. Interestingly, the development of pneumonia during the further course of AECOPD was retrospectively associated with significantly higher PSP/reg levels at admission (29.8 ng/mL [95% CI, 27.8-46.5] vs 23.8 ng/mL [95% CI, 17.1-32.7], $P < .01$).

PSP/reg level, age, Charlson age- and condition-related score, GOLD stage, Pco_2, and PCT guidance were associated with 2-year mortality on logistic regression analysis (Table 2). Kaplan-Meier analysis of the cumulative risk of death revealed increasing 2-year mortality for patients with low (< 25th percentile ≤ 18.4 ng/mL), intermediate (25-75th percentile = 18.4-33.9 ng/mL), and high PSP/reg levels (> 75th percentile ≥ 33.9 ng/mL) ($P < .01$) (Fig 1).

Sputum Bacteriology, Discolored Sputum, and PSP/reg

Sputum samples were obtained from 113 of 200 patients (56%). Sputum of five patients (7%) was of poor quality and therefore excluded from the analysis. Positive sputum cultures were found in 65 patients (56%). Microbiologic findings are presented in Table 3. Coinfection with more than one bacterial pathogen was diagnosed in seven patients (11%). Baseline characteristics depending on culture results showed similar clinical presentation, including the

Table 1—*Baseline Characteristics of Overall Study Population (N = 200) and 103 Patients With Positive or Negative Sputum Bacteriology Admitted to Hospital for AECOPD*

Characteristics	All (N = 200)	Positive Sputum Culture (n = 65)	Negative Sputum Culture (n = 43)	*P* Value
Epidemologic				
Age (range), y	70 (42-91)	70 (51-91)	69 (42-84)	.95
Male sex, %	114 (57)	47 (72)	20 (46)	.03
Weight, kg	67.1 (±15)	67.9 (±13)	71.3 (±16)	.39
Medical history				
Smoking, pack-y	45 (±28)	48 (±23)	42 (±24)	.50
Duration of COPD, mo	126 (±83)	125 (±77)	118 (±83)	.56
Duration AECOPD, d	6.9 (±10)	8.5 (±15)	4.6 (±4.5)	.09
AECOPD in previous years, %	154 (77)	49 (75)	35 (71)	.13
Hospitalization for AECOPD in previous year, %	102 (51)	32 (49)	25 (48)	.52
Type of AECOPD: Anthonisen criteria, %				
1: Increased dyspnea, sputum purulence, sputum volume	100 (50)	40 (61)	25 (58)	.06
2: Two of the above	44 (22)	11 (18)	11 (26)	.88
3: One of the above and more than one minor finding[a]	56 (28)	14 (21)	7 (16)	.13
GOLD stage and severity of COPD,[b] %				
I (FEV_1 > 80% predicted)	25 (12)	7 (11)	3 (7)	.21
II (50% predicted > FEV_1 % < 80% predicted)	42 (21)	12 (18)	11 (25)	.84
III (30% predicted > FEV_1 % < 50% predicted)	83 (42)	29 (45)	15 (35)	.03
IV (FEV_1 % predicted < 30% predicted)	50 (25)	17 (26)	14 (33)	.59
Lung function				
$FEV_{1\%}$ predicted	40 (±18.3)	43 (±18)	42 (±19)	.78
FEV_1, L	1.07 (±0.5)	1.09 (±0.5)	1.04 (±0.5)	.76
Blood gas analysis				
O_2 saturation, %	90 (±10)	91 (±9)	92 (±5)	.32
PaO_2, mm Hg	63.5 (±16)	62.9 (±9)	63.9 (±14)	.86
$PaCO_2$, mm Hg	43 (±11)	42 (±8.6)	43 (±8.6)	.43
Comorbidity (Charlson score)				
Weighted index	3.3 (±2.5)	2.7 (±1.9)	2.9 (±2.4)	.76
Condition- and age related score	6.0 (±2.7)	5.3 (±2.2)	5.4 (±2.9)	.92
Comorbidity, %				
Arterial hypertension	47 (24)	13 (20)	7 (16)	.63
Diabetes mellitus	23 (11)	7 (11)	7 (16)	.40
Ischemic heart disease	85 (42)	29 (45)	17 (40)	.61
Chronic renal failure	16 (8)	7 (11)	4 (9)	.81
Malignancy	26 (13)	5 (8)	3 (8)	.89
Vital signs				
Respiratory rate, breaths/min	24 (±6)	25 (±7)	22 (±5)	.18
Body temperature, °C	37.6 (±0.9)	37.5 (±0.9)	37.5 (±0.9)	.84
Respiratory symptoms, %				
Cough increased	173 (87)	60 (92)	39 (91)	.77
Sputum increased	139 (70)	51 (78)	34 (79)	.94
Discolored sputum	116 (58)	44 (68)	30 (70)	.82
Dyspnea increased	187 (93)	57 (88)	41 (95)	.18
Fever	83 (41)	29 (45)	18 (42)	.78
Treatment previous to hospital admission, %				
Antibiotic agent	43 (22)	13 (20)	11 (26)	.49
Oral steroids	66 (33)	26 (40)	9 (21)	.85
Inhaled steroids	144 (72)	47 (72)	38 (88)	.80
Long-acting β_2 agonists	170 (85)	55 (85)	39 (91)	.58
Long-acting anticholinergics	100 (50)	38 (59)	26 (60)	.97
Xanthine	22 (11)	4 (6)	2 (5)	.21
Long-term oxygen therapy	29 (15)	10 (15)	9 (21)	.90
In-hospital treatment, %				
Antibiotic agent	117 (56)	44 (68)	24 (56)	.21
Oral steroids	182 (87)	56 (86)	35 (81)	.51
Chemistry				
Leukocyte counts, $\times 10^9$/L	11.1 (±4.8)	11.7 (±4.7)	12.8 (±12.1)	.46

(Continued)

Table 1—*Continued*

Characteristics	All (N = 200)	Positive Sputum Culture (n = 65)	Negative Sputum Culture (n = 43)	*P* Value
CRP, mg/dL	35.1 (±46.4)	45.7 (±52.6)	46.4 (±63.2)	.37
PCT, ng/dL	0.26 (±0.8)	0.29 (±0.6)	0.14 (±0.12)	.27

Values are absolute numbers (%) or mean (SD) unless otherwise indicated. AECOPD = acute exacerbation of COPD; CRP = C-reactive protein; GOLD = Global Initiative for Chronic Obstructive Lung Disease; O_2 = oxygen; PCT = procalcitonin.

[a]Minor findings: fever (>38°C without any other cause), increased wheezing, increased cough, or increased respiratory rate compared with stable baseline condition.

[b]Classification of severity of COPD based on lung function tests performed at recovery (days 14-21 after admission),

frequency of discolored sputum (68% vs 70%, $P = .82$) and antibiotic prescriptions (68% vs 56%, $P = .21$) (Table 1).

Although PSP/reg levels showed some overlap between patients with positive and negative sputum cultures, positive sputum cultures were associated with higher PSP/reg levels (26.1 ng/mL; 95% CI, 19.2-38.1) compared with negative cultures in AECOPD (20.8 ng/mL; 95% CI, 15.6-27.2; $P < .01$) and control subjects with stable COPD (19.1 ng/mL; 95% CI, 14.1-30.4; $P < .01$) (Fig 2). PSP/reg levels were similar in culture-negative AECOPD and stable COPD (20.8 ng/mL; 95% CI, 15.6-27.2; $P = .83$). On a multivariate analysis, PSP/reg remained the only independent predictor of positive sputum bacteriology in AECOPD (OR, 6.59; 95% CI, 1.6-26.6; $P < .01$) (Table 4). Together with the FEV_1 % predicted, PSP/reg level remained an independent predictor of positive sputum bacteriology if the subgroups of patients who had not taken antibiotics and those receiving antibiotics at hospital admission were analyzed separately. The diagnostic performance of sputum color, PSP/reg level, and the combination of both to predict positive sputum cultures is depicted in Table 5. PSP/reg level showed a modest improvement in diagnostic performance (area under the curve, 0.65) compared with discolored sputum (area under the curve, 0.49). However, the combination of both performed significantly better than each single marker. PSP/reg > 18.4 ng/mL and presence of discolored sputum presented a sensitivity of 92% to predict positive sputum bacteriology. In other words, the absence of both conditions widely ruled out positive sputum cultures. The presence of both PSP/reg >33.9 ng/mL and discolored sputum predicted a positive bacterial sputum with 97% specificity.

Table 2—*Logistic Regression Analysis to Predict Mortality Within 2 Years After Hospitalization for AECOPD*

Variable	Logistic Regression Analysis		
	OR	95% CI	*P* Value
Age	1.05	1.01-1.09	.02
Weight	0.98	0.95-1.01	.14
Charlson score	1.47	1.24-1.74	<.01
GOLD stage	1.56	1.06-2.30	.03
FEV_1 predicted	0.96	0.97-1.02	.69
SaO_2 %	0.97	0.94-1.01	.18
Po_2	0.98	0.96-1.01	.24
Pco_2	1.06	1.03-1.11	<.01
PCT	1.50	1.08-2.7	.01
PSP/reg	2.77	2.77-5.41	<.01
CRP	1.07	0.86-1.34	.56

Charlson score = age- and condition-related score; PSP/reg = pancreatic stone protein/regenerating protein; SaO_2 = peripheral oxygen saturation. See Table 1 legend for expansion of other abbreviations.

Discussion

The present study is the first analyzing PSP/reg levels in COPD, to our knowledge. We revealed some interesting new findings. First, patients with positive sputum cultures presented significantly higher PSP/reg levels than those with negative sputum cultures at exacerbation and those with stable disease. This indicates that PSP/reg level might specifically reflect bacterial airway infection at exacerbation in contrast to chronic colonization in COPD. Second, the combination of both discolored sputum and PSP/reg > 18.4 ng/mL reliably allowed the identification of patients with positive sputum cultures, potentially offering guidance for antibiotic therapy at exacerbation. Third, PSP/reg level remained predictive of positive sputum cultures at exacerbation independently of the clinical characteristics, suggesting an additional value of this biomarker besides sputum color and clinical assessment. Fourth, elevated PSP/reg level was associated with increased 2-year mortality, strengthening the link between the benefit of antibiotic use at exacerbation and long-term survival.

PSP/reg has been proposed to act as an acute-phase protein.[20] PSP/reg is considered to be regulated by IL-6, tumor necrosis factor-α, and other cytokines released during inflammation and infection.[23] Although still underinvestigated, a role in bacterial infection is further supported by certain functional properties of PSP/reg. It was shown that PSP/reg itself, as well as the cleaved form, aggregate bacteria.[24] Moreover, in neutrophil granulocytes, PSP/reg induced a shedding

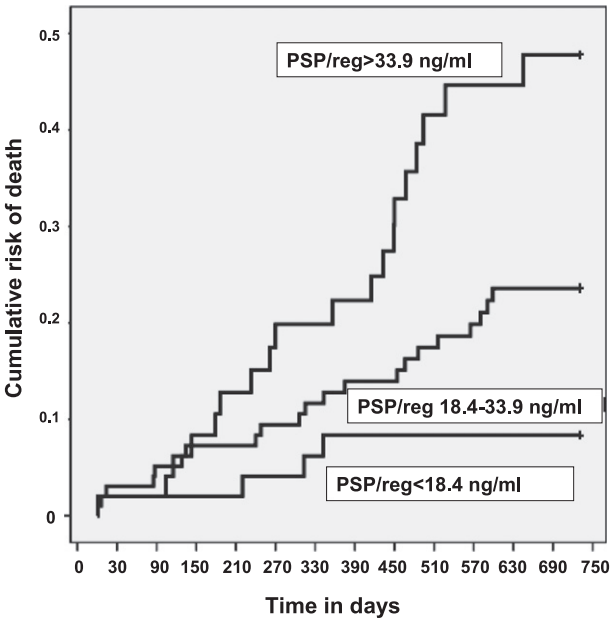

FIGURE 1. Kaplan-Meier analysis of cumulative risk of death according to PSP/reg quartiles at AECOPD. AECOPD = acute exacerbation in COPD; PSP/reg = pancreatic stone protein/regenerating protein.

of L-selectin and a downregulation of β_2-integrin, indicating neutrophil activation.[20] PSP/reg in peripheral blood might point toward a specific regulatory response leading to induction and activation of neutrophils and polymorphonuclear cells.[20] Thus, PSP/reg is a potential infection marker. In the current study, circulating PSP/reg levels allowed us to differentiate between patients with exacerbation and stable disease. More importantly, levels were significantly higher in subjects presenting with an acute exacerbation and positive sputum cultures compared with those with an acute exacerbation and negative sputum cultures. Interestingly, PSP/reg levels significantly decreased in patients with positive sputum cultures who received antibiotics at exacerbation. Therefore, it is tempting to hypothesize that higher levels of this biomarker at exacerbation indicates invasive or clinically relevant bacterial infection compared with either no bacteria or chronic bacterial colonization, which can be observed in ≤ 50% of patients with advanced, stable COPD.[25]

Sputum purulence is commonly assumed to be the clinical gold standard to identify bacterial exacerbation of COPD.[5,26] Remarkably, the association of sputum color with microbiologic analysis has been neither invariably replicated nor has it failed to predict bacteria on Gram stain or culture.[27] In addition, microbiologic examination of sputum specimens require several days to reveal bacterial growth and is only requested in about 10% of AECOPD.[28,29] Nevertheless, discolored sputum has been shown to be associated with heightened local and systemic airway inflammation.[30,31] In our analysis, discolored sputum performed poorly as a predictor of pathogenic bacterial growth in sputum (area under the curve, 0.49). However, the combination of discolored sputum and PSP/reg level at different cutoffs was capable of either widely ruling out (sensitivity, 92%) or confirming (specificity, 97%) positive bacterial sputum cultures. Considering that antibiotic treatment of viral infections or other noninfectious conditions increases the risk of drug toxicity and bacterial resistance,[32] the potential of the combination of discolored sputum and PSP/reg level to identify patients requiring antibiotic treatment of bacterial exacerbation might support an PSP/reg-guided approach for antibiotics at exacerbation. This issue should be evaluated in a properly designed, randomized, controlled trial.

The use of biomarkers to estimate the presence of bacterial infection and the response to antibiotic therapy is a relatively new approach to characterize differences in AECOPD and stable COPD.[10] PCT and CRP are the best studied biomarkers for this purpose.[13] Circulating PCT levels are higher in bacterial

Table 3—*Microbiologic Results of Positive Sputum Cultures at AECOPD (n = 65)*

Gram-Negative Bacteria	No. (%)	Gram-Positive Bacteria	No. (%)
Enterobacteriaceae species[a]	21 (29)	*Streptococcus pneumoniae*	11 (15)
Haemophilus species[b]	10 (14)	*Staphylococcus aureus*[e]	5 (7)
Pseudomonas species	8 (11)	MRSA	2 (2)
Moraxella catarrhalis[c]	4 (6)	…	…
Stenotrophomonas maltophilia[d]	4 (6)	…	…
Proteus species	4 (6)	…	…
Klebsiella species	3 (4)	…	…

MRSA = methicillin-resistant *Staphylococcus aureus*. See Table 1 legend for expansion of other abbreviations.

[a]Two cases with coinfection of enterobacteriaceae species and *Pseudomonas aeruginosa*

[b]Two cases with coinfection of *Haemophilus* species and *S pneumoniae*

[c]One case with coinfection of *M catarrhalis* and *S pneumoniae*

[d]One case with coinfection of *S maltophilia* and *Klebsiella* species.

[e]One case with coinfection of *S aureus* and enterobacteriaceae species.

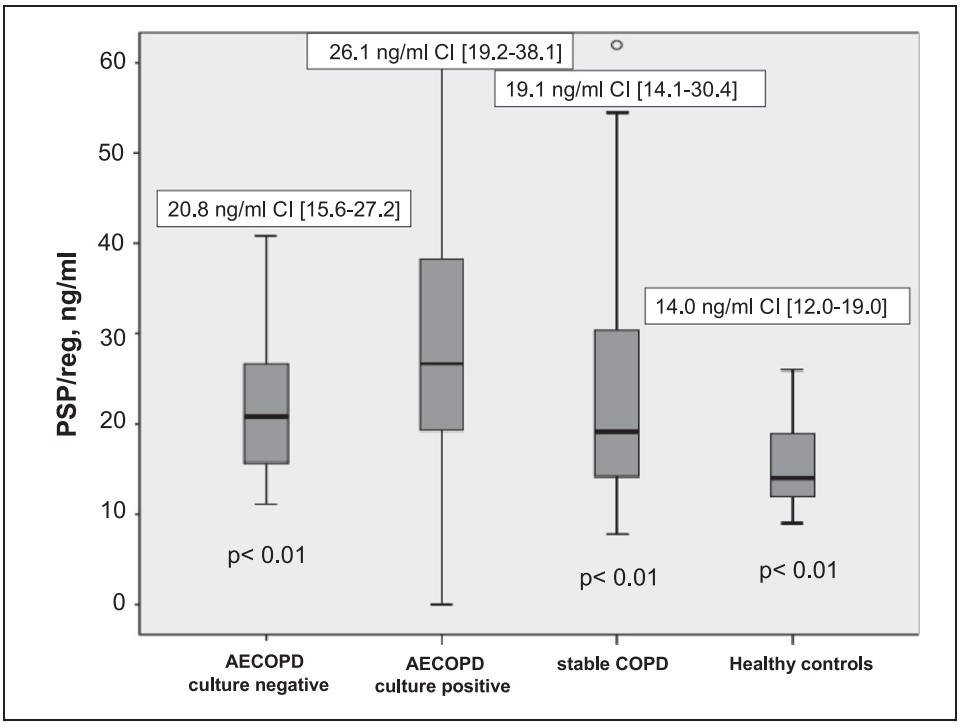

FIGURE 2. Median PSP/reg serum levels differed significantly in patients presenting with culture-positive compared with culture-negative AECOPD ($P < .01$), stable COPD ($P < .01$), and healthy control subjects ($P < .01$). See Figure 1 legend for expansion of abbreviations.

infection than in viral or other inflammatory conditions.[33] PCT is assumed to be highly specific for invasive bacterial infections like pneumonia and sepsis, which are not present in most patients with COPD.[33,34] According to recent investigations, there is no correlation between PCT and sputum cultures in AECOPD.[13,14] CRP has been invariably associated with discolored sputum.[35,36] Studies providing microbiologic analysis have not reached common conclusions because increased CRP levels in bacterial AECOPD have been described inconsistently.[14,37-39] However, CRP was considered to discriminate between positive and negative sputum cultures, leading to the conclusion that bacterial infection in AECOPD might be more accurately reflected by CRP than PCT.[36,37] Our results revealed no correlation of CRP and positive bacterial sputum cultures. Although we can only speculate about the reasons, previous data suggest that CRP values are probably associated with ≤ 50% interindividual variations caused by inherited characteristics and other confounders like steroid pretreatment, which may blunt CRP response by 50% to 63%.[40,41]

On multivariate analysis, PSP/reg level was an independent predictor of bacterial growth on sputum cultures. Thereby, PSP/reg level did not correlate with Anthonisen type 1 exacerbations, which were associated with greater likelihood of more severe exacerbations of bacterial origin.[5] Accordingly, recent studies failed to demonstrate a link between exacerbation severity and risk stratification based on Anthonisen criteria.[27]

In line with previous results, Charlson score, GOLD stage, hypercapnia, and PCT level were shown to have prognostic implications in our cohort of elderly

Table 4—*Logistic Regression Analysis to Predict Positive Sputum Bacteriology in AECOPD*

	Univariate Analysis			Multivariate Analysis		
Variable	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Charlson score	0.99	0.83-1.17	.86	0.84	0.68-1.03	.66
FEV_1 % predicted	1.03	1.01-1.05	<.01	1.03	0.99-1.05	.11
CRP	1.23	0.89-1.67	.28	1.15	0.94-1.34	.34
PCT	1.3	0.84-2.0	.32	1.03	0.99-1.05	.42
PSP/reg	3.57	1.35-9.44	<.01	6.59	1.64-26.6	<.01

See Table 1 and 2 legends for expansion of abbreviations.

Table 5—*Diagnostic Performance of PSP/reg to Predict Sputum Bacteriology in AECOPD*

Characteristics	Sensitivity	Specificity	PPV	NPV
Discolored sputum	67	30	59	38
PSP/reg > 18.4 ng/ml	79	37	65	55
PSP/reg > 33.9 ng/ml	33	93	87	49
Discolored sputum or PSP/reg > 33.9 ng/mL	79	26	61	46
Discolored sputum and PSP/reg > 18.4 ng/mL	92	10	60	44
Discolored sputum and PSP/reg > 33.9 ng/mL	22	97	93	35

Data given as %. NPV = negative predictive value; PPV = positive predictive value. See Table 1 and 2 legends for expansion of other abbreviations.

patients with advanced COPD and frequent prehospitalizations.[42-46] Patients with highest PSP/reg levels had a higher risk for death following hospital admission, supporting the notion that clinically relevant bacterial infection in AECOPD might result in greater illness and worse outcome. This observation is also in agreement with recent data of observational studies indicating that antibiotic therapy at exacerbation may improve long-term survival in COPD.[10,47] Besides its potential role in bacterial infection, pancreatic up-regulation of PSP/reg might occur due to several stimuli. Of note, we previously showed a fairly good correlation of PSP/reg with sequential organ-failure assessment scores at onset of ventilator-associated pneumonia. Sequential organ failure assessment is a frequently used measure in the ICU. Consequently, we believe that deterioration of distinct cells and organs cause PSP/reg elevations. This might explain why PSP/reg level is increased in patients with poor prognosis.

This study has a few limitations. In absence of a gold standard characterizing bacterial etiology of AECOPD, it remains, at best, difficult to demonstrate the diagnostic value of any parameter. Furthermore, due to its observational character, this study should be considered hypothesis generating. The potential role of PSP/reg level in an antibiotic-guided approach at exacerbation should be examined in a randomized, controlled trial. PSP/reg might represent a promising new biomarker to identify bacterial etiology of COPD exacerbation.

Acknowledgments

Author contributions: Dr Scherr had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Dr Scherr: contributed to study conception and design and drafting the manuscript for important intellectual content, read and approved the final manuscript, and served as principal author.
Dr Graf: contributed to data analysis and interpretation and drafting the manuscript for important intellectual content, read and approved the final manuscript, and takes responsibility for the integrity of the work as a whole.
Ms Bain: contributed to data analysis and interpretation and drafting the manuscript for important intellectual content, read and approved the final manuscript, and takes responsibility for the integrity of the work as a whole.
Dr Christ-Crain: contributed to data analysis and interpretation and drafting the manuscript for important intellectual content, read and approved the final manuscript, and takes responsibility for the integrity of the work as a whole.
Dr Müller: contributed to data analysis and interpretation and drafting the manuscript for important intellectual content, read and approved the final manuscript, and takes responsibility for the integrity of the work as a whole.
Dr Tamm: contributed to contributed to study conception and design, data analysis and interpretation, and drafting the manuscript for important intellectual content; read and approved the final manuscript; and takes responsibility for the integrity of the work as a whole.
Dr Stolz: contributed to contributed to study conception and design, data analysis and interpretation, and drafting the manuscript for important intellectual content; read and approved the final manuscript; and takes responsibility for the integrity of the work as a whole.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Role of sponsors: The sponsors of this investigator-initiated project had no involvement in the design or conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, and approval of the manuscript; or decision to submit the manuscript.

References

1. Garcia-Aymerich J, Escarrabill J, Marrades RM, Monsó E, Barreiro E, Antó JM; EFRAM Investigators. Differences in COPD care among doctors who control the disease: general practitioner vs. pneumologist. *Respir Med*. 2006;100(2):332-339.
2. Fuso L, Incalzi RA, Pistelli R, et al. Predicting mortality of patients hospitalized for acutely exacerbated chronic obstructive pulmonary disease. *Am J Med*. 1995;98(3):272-277.
3. Papi A, Luppi F, Franco F, Fabbri LM. Pathophysiology of exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2006;3(3):245-251.
4. White AJ, Gompertz S, Stockley RA. Chronic obstructive pulmonary disease. 6: The aetiology of exacerbations of chronic obstructive pulmonary disease. *Thorax*. 2003;58(1):73-80.
5. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med*. 1987;106(2):196-204.
6. Saint S, Bent S, Vittinghoff E, Grady D. Antibiotics in chronic obstructive pulmonary disease exacerbations. A meta-analysis. *JAMA*. 1995;273(12):957-960.
7. McCrory DC, Brown C, Gelfand SE, Bach PB. Management of acute exacerbations of COPD: a summary and appraisal of published evidence. *Chest*. 2001;119(4):1190-1209.
8. Berry DG, Fry J, Hindley CP, et al. Exacerbations of chronic bronchitis treatment with oxytetracycline. *Lancet*. 1960;1(7116):137-139.
9. Nicotra MB, Rivera M, Awe RJ. Antibiotic therapy of acute exacerbations of chronic bronchitis. A controlled study using tetracycline. *Ann Intern Med*. 1982;97(1):18-21.
10. Rothberg MB, Pekow PS, Lahti M, Brody O, Skiest DJ, Lindenauer PK. Antibiotic therapy and treatment failure in patients hospitalized for acute exacerbations of chronic obstructive pulmonary disease. *JAMA*. 2010;303(20):2035-2042.

11. Albrich WC, Müller B, Harbarth S. Antibiotic therapy in patients hospitalized with acute chronic obstructive pulmonary disease. *JAMA*. 2010;304(12):1326.
12. Ram FS, Rodriguez-Roisin R, Granados-Navarrete A, Garcia-Aymerich J, Barnes NC. Antibiotics for exacerbations of chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. 2006; (2):CD004403.
13. 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(1):9-19.
14. Daniels JM, Schoorl M, Snijders D, et al. Procalcitonin vs C-reactive protein as predictive markers of response to antibiotic therapy in acute exacerbations of COPD. *Chest*. 2010;138(5):1108-1115.
15. Watanabe T, Yonekura H, Terazono K, Yamamoto H, Okamoto H. Complete nucleotide sequence of human reg gene and its expression in normal and tumoral tissues. The reg protein, pancreatic stone protein, and pancreatic thread protein are one and the same product of the gene. *J Biol Chem*. 1990;265(13):7432-7439.
16. Iovanna JL, Dagorn JC. The multifunctional family of secreted proteins containing a C-type lectin-like domain linked to a short N-terminal peptide. *Biochim Biophys Acta*. 2005; 1723(1-3):8-18
17. Graf R, Schiesser M, Reding T, et al. Exocrine meets endocrine: pancreatic stone protein and regenerating protein—two sides of the same coin. *J Surg Res*. 2006;133(2):113-120.
18. Bimmler D, Schiesser M, Perren A, et al. Coordinate regulation of PSP/reg and PAP isoforms as a family of secretory stress proteins in an animal model of chronic pancreatitis. *J Surg Res*. 2004;118(2):122-135.
19. Boeck L, Graf R, Eggimann P, et al. Pancreatic stone protein: a marker of organ failure and outcome in ventilator-associated pneumonia. *Chest*. 2011;140(4):925-932.
20. Keel M, Härter L, Reding T, et al. Pancreatic stone protein is highly increased during posttraumatic sepsis and activates neutrophil granulocytes. *Crit Care Med*. 2009;37(5):1642-1648.
21. Fabbri LM, Hurd SS; GOLD Scientific Committee. Global Strategy for the Diagnosis, Management and Prevention of COPD: 2003 update. *Eur Respir J*. 2003;22(1):1-2.
22. Isenberg HD. Clinical microbiology: past, present, and future. *J Clin Microbiol*. 2003;41(3):917-918.
23. Dusetti NJ, Ortiz EM, Mallo GV, Dagorn JC, Iovanna JL. Pancreatitis-associated protein I (PAP I), an acute phase protein induced by cytokines. Identification of two functional interleukin-6 response elements in the rat PAP I promoter region. *J Biol Chem*. 1995;270(38):22417-22421.
24. Iovanna J, Frigerio JM, Dusetti N, Ramare F, Raibaud P, Dagorn JC. Lithostathine, an inhibitor of CaCO3 crystal growth in pancreatic juice, induces bacterial aggregation. *Pancreas*. 1993;8(5):597-601.
25. Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA. Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. *Thorax*. 2002;57(9):759-764.
26. Soler N, Agustí C, Angrill J, Puig De la Bellacasa J, Torres A. Bronchoscopic validation of the significance of sputum purulence in severe exacerbations of chronic obstructive pulmonary disease. *Thorax*. 2007;62(1):29-35.
27. van der Valk P, Monninkhof E, van der Palen J, Zielhuis G, van Herwaarden C, Hendrix R. Clinical predictors of bacterial involvement in exacerbations of chronic obstructive pulmonary disease. *Clin Infect Dis*. 2004;39(7):980-986.
28. Woodhead M, Gialdroni Grassi G, Huchon GJ, Léophonte P, Manresa F, Schaberg T. Use of investigations in lower respiratory tract infection in the community: a European survey. *Eur Respir J*. 1996;9(8):1596-1600.
29. Sethi S. Infectious etiology of acute exacerbations of chronic bronchitis. *Chest*. 2000;117(5 suppl 2):380S-385S.
30. Allegra L, Blasi F, Diano P, et al. Sputum color as a marker of acute bacterial exacerbations of chronic obstructive pulmonary disease. *Respir Med*. 2005;99(6):742-747.
31. Stockley RA, Bayley D, Hill SL, Hill AT, Crooks S, Campbell EJ. Assessment of airway neutrophils by sputum colour: correlation with airways inflammation. *Thorax*. 2001;56(5):366-372.
32. Evans HL, Lefrak SN, Lyman J, et al. Cost of Gram-negative resistance. *Crit Care Med*. 2007;35(1):89-95.
33. Becker KL, Nylén ES, White JC, Müller B, Snider RH Jr. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. *J Clin Endocrinol Metab*. 2004;89(4):1512-1525.
34. Niederman MS. Biological markers to determine eligibility in trials for community-acquired pneumonia: a focus on procalcitonin. *Clin Infect Dis*. 2008;47(suppl 3):S127-S132.
35. Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD. *Chest*. 2000;117(6):1638-1645.
36. Weis N, Almdal T. C-reactive protein—can it be used as a marker of infection in patients with exacerbation of chronic obstructive pulmonary disease? *Eur J Intern Med*. 2006; 17(2):88-91.
37. Dev D, Wallace E, Sankaran R, et al. Value of C-reactive protein measurements in exacerbations of chronic obstructive pulmonary disease. *Respir Med*. 1998;92(4):664-667.
38. Bozinovski S, Hutchinson A, Thompson M, et al. Serum amyloid a is a biomarker of acute exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177(3):269-278.
39. Creutzberg EC, Wouters EF, Vanderhoven-Augustin IM, Dentener MA, Schols AM. Disturbances in leptin metabolism are related to energy imbalance during acute exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2000;162(4 Pt 1):1239-1245.
40. Wouters EF. The systemic face of airway diseases: the role of C-reactive protein. *Eur Respir J*. 2006;27(5):877-879.
41. Sin DD, Lacy P, York E, Man SF. Effects of fluticasone on systemic markers of inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2004;170(7): 760-765.
42. Gray-Donald K, Gibbons L, Shapiro SH, Macklem PT, Martin JG. Nutritional status and mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1996; 153(3):961-966.
43. Almagro P, Calbo E, Ochoa de Echagüen A, et al. Mortality after hospitalization for COPD. *Chest*. 2002;121(5):1441-1448.
44. Groenewegen KH, Schols AM, Wouters EF. Mortality and mortality-related factors after hospitalization for acute exacerbation of COPD. *Chest*. 2003;124(2):459-467.
45. Antonelli Incalzi R, Fuso L, De Rosa M, et al. Co-morbidity contributes to predict mortality of patients with chronic obstructive pulmonary disease. *Eur Respir J*. 1997;10(12): 2794-2800.
46. Stolz D, Christ-Crain M, Morgenthaler NG, et al. Plasma pro-adrenomedullin but not plasma pro-endothelin predicts survival in exacerbations of COPD. *Chest*. 2008;134(2):263-272.
47. Roede BM, Bresser P, Prins JM, Schellevis F, Verheij TJ, Bindels PJ. Reduced risk of next exacerbation and mortality associated with antibiotic use in COPD. *Eur Respir J*. 2009; 33(2):282-288.