CHEST

Systemic Biomarkers of Collagen and Elastin Turnover Are Associated With Clinically Relevant Outcomes in COPD

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BACKGROUND: Extracellular matrix (ECM) remodeling of the lung tissue releases protein fragments into the blood, where they may be detected as serologic surrogate markers of disease activity in COPD. Our goal was to assess the association of ECM turnover with severity and outcome of COPD.

METHODS: In a prospective, observational, multicenter study including 506 patients with COPD (Global Initiative for Chronic Obstructive Lung Disease grades II to IV), serum samples were analyzed at stable state, exacerbation, and 4 weeks after exacerbation. The analysis comprised a panel of five novel neoepitopes, including fragments of collagen type III (C3M) and collagen type VI (C6M), pro-forms of collagen type III (Pro-C3) and type VI (Pro-C6), and neutrophil elastase-generated fragments of elastin (EL-NE) according to enzyme-linked immunosorbent assay. These neoepitopes were also measured at stable state in a derivation cohort that included 100 patients with COPD.

RESULTS: Serum levels of C3M, C6M, Pro-C3, Pro-C6, and EL-NE were associated with lung function. Patients with the lowest levels of Pro-C3 and Pro-C6 had more severe airflow limitation, hyperinflation, air trapping, and emphysema. C3M and C6M were associated with dyspnea. All ECM biomarkers, except Pro-C6, were increased at exacerbation compared with stable state but, except EL-NE, did not differ between stable state and exacerbation follow-up in the crude and adjusted analyses. In Cox regression adjusted analyses, Pro-C3 was associated with a shorter time to exacerbation (hazard ratio, 0.72; CI, 0.59-0.89; $P = .002$) and Pro-C6 with survival (hazard ratio, 2.09; CI, 1.18-3.71; $P = .011$).

CONCLUSIONS: Serum biomarkers of ECM turnover were significantly associated with disease severity and clinically relevant outcomes in patients with COPD.

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ABBREVIATIONS: AECOPD = acute exacerbations of COPD; BALF = BAL fluid; C3M = fragments of collagen type III; C6M = fragments of collagen type VI; ECM = extracellular matrix; EL-NE = neutrophil elastase-generated fragments of elastin; GOLD = Global Initiative for Chronic Obstructive Lung Disease; ICS = inhaled corticosteroids; MMP = matrix metalloproteinase; MMRC = Medical Research Council Score; Pro-C3 = pro-forms of collagen type III; Pro-C6 = pro-forms of collagen type VI; SF-36 = 36-item Short-Form Health Survey; SGRQ = St. George's Respiratory Questionnaire; TIMP = tissue inhibitor of metalloproteinases

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COPD is a chronic inflammatory respiratory condition, characterized by progressive airflow limitation that is not fully reversible. The accelerated deterioration of pulmonary function may occur at any stage in the course of the disease. The associated episodes are called acute exacerbations of COPD (AECOPD). Frequent exacerbations hasten lung function decline, affecting quality of life, exercise capacity, and survival in patients with COPD.[1]

Although exacerbations are key events in the progression of COPD, little information is available regarding the mechanism by which exacerbations may contribute to airway remodeling and associated structural changes in lung tissue. In this respect, it has been shown that chronic inflammation in COPD and exacerbations are associated with disturbances in the homeostasis of extracellular matrix (ECM) molecules.[2,3] The ECM of the lung consists mainly of collagens, elastin, and proteoglycans, and it is subjected to a daily turnover of approximately 10%, indicating that subtle changes in turnover rates lead to large changes in total ECM composition with time. The interstitial matrix is the main local area affected by inflammation and is mainly composed of collagen type I and collagen type III as well as collagen type VI.[4-8] The main protein of the lung is elastin, which is responsible for the elastic properties of the lung tissue. It has been found that the remodeling of elastin in healthy states is very low, with a half-life of approximately 15 years; however, this level seems to be elevated in pulmonary diseases.[9,10]

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Matrix metalloproteinase (MMP)-2 and -9 have been implicated in the degradation of ECM components such as collagen type I, collagen type III, collagen type VI, as well as elastin, and are expressed by inflammatory cells such as macrophages.[8-12] With tissue inflammation, MMP expression and proteolytic activity are upregulated, causing protein fragments to be released into the circulation, where they may serve as ECM-specific biomarkers. Assessment of lung ECM protein fragments, also known as neoepitopes, may therefore provide novel biomarkers for disease exacerbation, prognosis, and activity.

We recently investigated key ECM molecules of the lung, such as MMPs, tissue inhibitors of metalloproteinases (TIMPs), and glycosaminoglycans. We found that levels of MMP-9, TIMP-1, TIMP-2, heparan sulfate, and chondroitin sulfate were significantly increased in BAL fluid (BALF) of patients with COPD during exacerbations.[13,14] Levels of heparan sulfate and chondroitin sulfate are significantly correlated with MMP-9, MMP-2, and MMP-12 in BALF, indicating that they were cleaved from their respective proteoglycans by MMPs and subsequently washed out in BALF. Moreover, a significant turnover of hyaluronic acid was observed in BALF during exacerbations.[15] These data indicate that during AECOPD, there is an increased turnover of ECM molecules, which may be associated with airway remodeling and lung function decline during exacerbations of COPD.

In the present study, we hypothesized that systemic biomarkers of ECM protein degradation and formation, reflecting ECM turnover, are increased during COPD exacerbations and that ECM activity is associated with disease severity and outcome in COPD.

Materials and Methods

Study Design and Patients

Data from two independent cohorts were analyzed in the present study. The confirmation cohort consisted of 638 patients enrolled in the Predicting Outcome Using Systemic Markers in Severe Exacerbations of Chronic Obstructive Pulmonary Disease (PROMISE-COPD) trial, a multicenter study in 11 centers in eight European countries that evaluated the potential predictors of outcome in patients with moderate to very severe COPD. The PROMISE-COPD study was designed to be inclusive, exploratory, and hypothesis-generating, and it was specifically drafted to identify predictors of outcome by using systemic markers in COPD. All enrolled patients had an initial baseline examination at stable state and were followed up for at least 2 years in scheduled half yearly visits. When necessary, patients underwent outpatient visits or were hospitalized for treatment of AECOPD, and follow-up visits were performed 4 weeks after the onset of exacerbation. Throughout the

study duration, patients were treated as clinically warranted, without restriction. Patients were monitored for recurrent moderate (requiring treatment with systematic corticosteroids, antibiotics, or both) and severe (requiring hospitalization or a visit to the ED) AECOPD. Of the 638 patients, 506 attended a scheduled visit at 6 months and were therefore eligible for inclusion in the subsequent analyses. Clinical history, physical examinations, lung function, and 6-min walking test were performed for each patient.[16] The age-adjusted Charlson Comorbidity Index score was also calculated for patients included in the confirmation cohort. Each patient completed the following: the Modified Medical Research Council Score (MMRC); the St. George's Respiratory Questionnaire (SGRQ) COPD version; and the 36-item Short-Form Health Survey (SF-36), a health-related, quality of life questionnaire. All examinations took place at each scheduled visit (semi-annually), except for the 6-min walking test. At the 2-year follow-up, the vital status of each patient was confirmed.

The derivation cohort consisted of 100 patients included in the Basel Study on COPD (BASCO), a cross-sectional, observational, monocentric study that included patients with COPD undergoing bronchoscopy for clinical indications. Serum samples, at stable state, were collected at the day of bronchoscopy. In the confirmation cohort, serum samples were collected at stable state, at exacerbation, and at exacerbation follow-up (4 weeks after exacerbation).

A control group was also included that consisted of 56 unselected, consecutive healthy blood donors. Serum samples from the control group were treated identically with serum samples from the derivation and the confirmation cohorts.

For both studies, patients with COPD were categorized as Global Initiative for Chronic Obstructive Lung Disease (GOLD) grade II to IV. BASCO and PROMISE-COPD were investigator-initiated and -driven studies, compiled according to the Declaration of Helsinki and Good Clinical Practice Guidelines, and were approved by the institutional review board (EKBB05/06 and EKBB295/07). The PROMISE-COPD study was registered at www.controlled-trials.com (identifier ISRCTN99586989). All patients provided written consent before initiation of any study assessments. A detailed description of the two cohorts is provided in e-Appendix 1.

Determination of Biomarkers

Serum levels of fragments of collagen type III (C3M) and collagen type VI (C6M) pro-forms of collagen type III (Pro-C3) and type VI (Pro-C6), and neutrophil elastase-generated fragments of elastin (EL-NE) were measured by using Nordic Bioscience assays according to the manufacturer's instructions.[17] All assays used a monoclonal antibody directed against either a protein fragment produced by protease cleavage during degradation or formation, or an internal protein sequence, as described previously.[17] All serum samples were assayed in duplicate, in a single run by personnel unaware of the patients' clinical data. Values of biomarkers were divided into quartiles for data analyses.

Statistics

Continuous variables are expressed as mean ± SD or median (interquartile range [25th-75th percentile]), and discrete variables as percentages. The associations between biomarkers of ECM turnover and clinical parameters were evaluated by univariable linear regression models including MMRC, FEV_1 % predicted postbronchodilator, FEV_1/FVC, oxygen saturation at rest and lowest oxygen saturation at exercise, distance at the 6-min walking test, and health-related quality of life (SF-36 and SGRQ). To analyze the level of different biomarkers over time, linear mixed-effects models were performed. Analyses were conducted, both unadjusted and adjusted, for the clinical characteristics such as sex, BMI, FEV_1 % predicted, MMRC, age-adjusted Charlson Comorbidity Index score, and log10-transformed biomarker values at stable state. To predict the risk of exacerbation or death from the biomarker values at stable state over time, Cox regression models were performed. Analyses were adjusted according to sex, BMI, FEV_1 % predicted, MMRC, and age-adjusted Charlson Comorbidity Index score. Hazard ratios indicate increment of risk attributed to the doubling of the biomarker (log2 scale). Statistical analyses were performed by using R version 3.1.3 (R Foundation for Statistical Computing) and SPSS version 22.0.

Results

ECM Biomarkers in Patients With COPD

In the PROMISE-COPD cohort, most patients were male and had a considerable smoking history, clinically relevant disease (1 exacerbation requiring physician attention in the previous year) (Table 1), and multiple comorbidities (e-Table 1). Patients were followed up for a median of 722 days (interquartile range, 395-762 days). During the follow-up, 317 patients (62.6%) experienced ≥ 1 exacerbation (354 exacerbations in total), and 38 patients died (7.5%) (Fig 1). Characteristics of the 354 exacerbations are presented in Table 2. The demographic characteristics of the 100 patients included in the derivation cohort in stable state are depicted in e-Table 2.

We first investigated the association between the circulating biomarkers of ECM turnover and severity of airflow limitation as assessed according to GOLD grades. In the PROMISE-COPD cohort, a significant association was observed between collagen type III and collagen type VI turnover and disease severity (Table 3). These data confirmed the observations obtained in the BASCO-COPD cohort (e-Table 3).

We further investigated the association of the circulating biomarkers of ECM turnover with lung function as assessed by FEV_1 % predicted and FEV_1/FVC. In the PROMISE-COPD cohort, C3M, C6M, Pro-C3, Pro-C6, and EL-NE were associated with the FEV_1 % predicted (Table 4). Similarly, there was an association of Pro-C3 and Pro-C6 with airflow limitation as expressed by the FEV_1/FVC. Most of the aforementioned associations were also evident in the BASCO-COPD cohort (e-Table 4).

In the PROMISE-COPD cohort, patients with lower levels of Pro-C3 had more severe airflow limitations (FEV_1 and FEV_1/FVC), hyperinflation (total lung capacity), air trapping (residual volume/total lung capacity), and emphysema (diffusion capacity) compared with patients with higher circulating levels

TABLE 1] Characteristics of 506 Patients With COPD GOLD II-IV Included in the Stable State in the PROMISE-COPD Cohort

Characteristic	Value (N = 506)
Age, y	66.8 ± 10.5
Male	366 (71.9)
BMI, kg/m^2	26.2 ± 5.5
Weight, kg	74.9 ± 17.0
Height, cm	169.0 ± 8.0
White race, %	501 (99.0)
COPD history	
Current smoker	150 (29.6)
Pack-years, y	51.5 ± 30.9
Duration of COPD symptoms, mo	102.7 ± 89.1
Time elapse since diagnosis, mo	80.2 ± 74.6
No. of exacerbations in previous year	1 (0-1)
No. of severe exacerbations in previous year	0 (0-1)
MMRC dyspnea scale	1 (1-2)
BODE index	3 (1-4)
6MWD, m	380.3 ± 104.2
Borg score	4 (3-6)
Peripheral oxygen saturation at rest, %	94.5 ± 2.7
Lowest peripheral oxygen saturation at exercise, %	89.5 ± 5.7
SGRQ	
Symptoms score	49.0 ± 22.7
Activity score	57.4 ± 22.8
Impact score	32.2 ± 18.7
Total score	42.4 ± 18.1
SF-36	
Physical function	51.4 ± 25.9
Role physical	51.7 ± 43.5
Role emotional	67.5 ± 42.9
Social functioning	69.8 ± 28.2
Mental health	65.4 ± 19.8
Body pain	73.9 ± 27.6
Vitality	51.9 ± 20.9
General health	48.2 ± 23.1
GOLD grade[a]	
II	253 (50.0)
III	177 (35.0)
IV	76 (15.0)
Lung function parameters	
FVC, post-brd, % predicted	77.8 ± 24.8
FEV_1, post-brd, % predicted	48.6 ± 18.2
FEV_1/FVC post-brd, %	48.2 ± 14.1
Residual volume, %[b]	157.4 ± 45.6
Total lung capacity, %[b]	118.8 ± 20.2
Residual volume/total lung capacity, %[b]	53.7 ± 9.6
Diffusion capacity, %[b]	55.6 ± 20.7
ABGA	
Oxygen pressure, kPa[b]	9.2 ± 6.9
CO_2 pressure, kPa[b]	5.3 ± 0.9

(Continued)

TABLE 1] *(Continued)*

Continuous data are shown as mean ± SD or median (interquartile range), and categorical variables as No. (%). 6MWD = 6-min walk distance; ABGA = arterial blood gas analysis; BODE = BMI, airflow obstruction, dyspnea, and exercise capacity; brd = bronchodilator; GOLD = Global Initiative for Chronic Obstructive Lung Disease; MMRC = modified Medical Research Council; PROMISE-COPD = Predicting Outcome Using Systemic Markers in Severe Exacerbations of Chronic Obstructive Pulmonary Disease; SF-36 = 36-item Short-Form Health Survey; SGRQ = St. George's Respiratory Questionnaire.

[a]GOLD grades are based on FEV_1 % predicted: II, ≥ 50% <80%; III, ≥ 30% < 50%; and IV, ≤ 30%.

[b]Results of body plethysmography (residual volume, total lung capacity, residual volume/total lung capacity, and diffusion capacity), as well as ABGA (oxygen pressure and CO_2 pressure), were collected for 257 patients of a predefined nested cohort.

of Pro-C3 (Fig 2A). Patients with lower levels of Pro-C6 had more severe hyperinflation, air trapping, and emphysema (Fig 2B). These observations were also reported in the BASCO-COPD cohort (data not shown).

We further analyzed the effect of inhaled corticosteroids (ICS) on epitope concentrations in the PROMISE-COPD cohort. C3M, C6M, and EL-NE were significantly higher ($P = .004$, $P = .010$, and $P = .040$, respectively) and Pro-C3 was significantly lower ($P = .047$) in patients receiving ICS compared with patients who did not receive ICS. However, when a linear regression analysis was performed (adjusted for FEV_1 % predicted), we found that only C6M remained significantly higher ($P = .02$) in patients receiving ICS.

ECM Biomarkers and Disease Impact

To investigate the influence of ECM biomarkers on disease impact, we evaluated their levels according to the degree of dyspnea as assessed by using the MMRC. In the PROMISE-COPD cohort, C3M, C6M, and EL-NE levels differed significantly according to the degree of dyspnea (geometric mean ratio, 1.25 [1.13-1.14], 1.38 [1.18-1.62], and 1.47 [1.14-1.90], respectively; all, $P < .0001$). C6M and Pro-C6 were associated with the exercise capacity (geometric mean ratios of 0.999

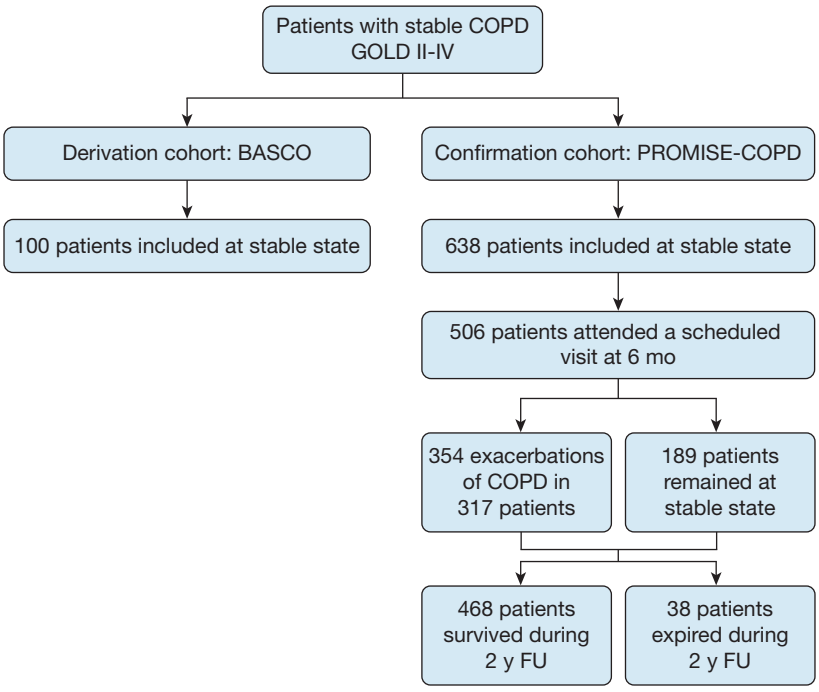

Figure 1 – *Study design according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. BASCO = Basel Study on COPD; GOLD = Global Initiative for Chronic Obstructive Lung Disease; FU = follow-up; PROMISE-COPD = Predicting Outcome Using Systemic Markers in Severe Exacerbations of Chronic Obstructive Pulmonary Disease.*

[0.999-1.000] [$P = .02$] and 0.999 [0.999-1.000] [$P < .001$]). Moreover, C6M exhibited an association with the health-related quality of life physical function domain of the SF-36 (geometric mean ratio, 0.998 [0.996-1.000]; $P = .02$).

Overall, Pro-C3 presented the strongest association with the health-related quality of life (SGRQ) and physical function domain of the SF-36 according to the distribution of Pro-C3 in quartiles (Q_1 vs Q_2-Q_4), at stable state (Fig 3, Table 5). Patients with the lowest levels of Pro-C3 had the poorest health-related quality of life in all the domains as assessed by using the SGRQ and the worst physical function in the SF-36.

ECM Biomarkers and Bacterial Colonization

In the PROMISE-COPD cohort, 155 patients (30.6%) at the stable state visit were able to produce sputum of good quality for microbiologic analysis. Forty-eight of these samples (30.9%) demonstrated potentially pathogenic bacteria growth. These patients, with verified bacterial colonization in sputum, had elevated C3M (33.2 ng/mL [26.9-39.6] vs 28.9 ng/mL [24.3-33.6]; $P = .044$), C6M (16.0 ng/mL [12.4-20.7] vs 12.3 ng/mL [10.2-17.3]; $P = .009$), and EL-NE (6.3 ng/mL [4.95-10.4] vs 4.8 ng/mL [1.6-9.0]; $P = .009$) compared with patients with negative sputum cultures.

ECM Biomarkers at Exacerbation

We further investigated if serum levels of ECM biomarkers were higher in patients with COPD compared with non-COPD control subjects. As shown in Table 6, levels of C3M, C6M, Pro-C6, EL-NE, Pro-C3/C3M, and Pro-C6/C6M were higher in patients with COPD compared with the control subjects.

To investigate if circulating levels of ECM biomarkers were altered between stable state and exacerbations of COPD, we measured their serum levels in patients of the PROMISE-COPD cohort at stable state, at exacerbation, and at 4 weeks after exacerbation (Fig 4). Except for Pro-C3, ECM biomarkers differed significantly between stable state and exacerbation in the crude and adjusted analyses (C3M, $P = 0.02$; all others, $P < .001$). Four weeks after exacerbation, values for C3M, C6M, and Pro-C6 returned to values obtained at stable state of the disease. EL-NE remained increased 4 weeks after exacerbation in the crude and adjusted analyses ($P = .002$ and $P = .005$). After adjustment for the clinical model (ie, sex, BMI, FEV_1 % predicted), circulating levels of C6M differed between exacerbation follow-up and stable state ($P = .04$).

Upon dichotomization, C6M, Pro-C6, and EL-NE were increased both in the moderate and severe exacerbations compared with the stable state before and after adjustment. C3M was consistently increased only in severe exacerbations. Pro-C3 was not associated with moderate or severe exacerbations in the crude and adjusted analyses.

Degradation and formation balance of collagen type VI (Pro-C6/C6M) differed at stable state and exacerbation,

TABLE 2] Characteristics of 354 Exacerbations of COPD in Patients With COPD GOLD II to IV Included in the PROMISE-COPD Cohort

Characteristics of Exacerbations	N = 354
Symptoms of exacerbation	
Increased dyspnea, %	335 (94.6)
Increased sputum volume, %	222 (63.0)
Increased sputum purulence, %	195 (55.9)
Sputum volume, % (n = 275)	
Small amount (1 teaspoon)	97 (35.3)
Moderate amount (1 soup spoon)	124 (45.1)
Large amount (> 1 soup spoon)	54 (19.6)
Sputum color, % (n = 264)	
Transparent	7 (2.7)
White	71 (26.9)
Purulent yellow	123 (46.6)
Purulent green	63 (23.9)
Duration of symptoms prior to visit,	6.4 ± 7.5
Chest radiograph and/or chest CT scan, %	207 (58.5)
Sputum microbiology	
Sputum collected, %	180 (51.6)
Culture performed, %	153 (93.9)
Potentially pathogenic microorganisms, %	82 (53.6)
Treatment of exacerbation[a]	
Combination of inhaled beta$_2$-agonists and anticholinergics, %	140 (39.4)
Inhaled steroids, %	109 (30.7)
Oral, IM or IV glucocorticosteroids, %	274 (77.3)
Oral or IV antibiotics, %	230 (65.0)
Oral or IV methylxanthines, %	29 (8.1)
Oxygen, %	100 (28.4)
New prescription or increased dosage of diuretic therapy, %	16 (5.95)
Severity of exacerbation	
Patients requiring hospitalization, no. %	117 (33.1)
Length of hospital stay, d	7 (4-10)
Use of accessory respiratory muscles, %	68 (20.7)
Paradoxical chest wall movements, %	2 (0.6)
Worsening or new-onset central cyanosis, %	13 (3.9)
Development of peripheral edema, %	17 (5.1)
Hemodynamic instability, %	4 (1.2)
Signs of right-heart failure, %	4 (1.2)
Reduced alertness, %	14 (4.1)
Noninvasive mechanical ventilation, %	9 (3.4)
Invasive mechanical ventilation, %	2 (0.8)
Indications for hospital admission for COPD exacerbation (n = 115)	
Marked increase in intensity of symptoms, such as sudden development of resting dyspnea, %	88 (76.1)
Severe underlying COPD, %	87 (75.4)
Onset of new physical signs (eg, cyanosis, peripheral edema), %	25 (21.9)
Failure of exacerbation to respond to initial medical management, %	49 (42.5)
Significant comorbidities, %	27 (23.2)
Frequent exacerbations, %	1 (0.9)
Newly occurring arrhythmias, %	1 (0.9)
Diagnostic uncertainty, %	5 (4.4)
Older age, %	49 (42.6)
Insufficient home support, %	18 (15.7)

Continuous data are shown as mean ± SD or median (interquartile range), and categorical variables as No. (%). See Table 1 legend for expansion of abbreviations.

[a]Percentages refer to the number of available observations and thus do not always add to 354.

both moderate and severe, before and after adjustment for the clinical model ($P < .001$ for all). In contrast, the turnover of collagen III (Pro-C3/C3M) was similar at stable state and exacerbation in all models.

ECM Biomarkers and Disease Outcome

Patients experiencing an exacerbation during the 2-year follow-up had significantly lower levels of Pro-C3 and Pro-C6 ($P < .001$ and $P = .001$) and EL-NE ($P = .041$) at stable state compared with patients who did not have an exacerbation during the 2-year follow-up (Fig 5A). Levels for C3M and C6M did not differ between survivors and nonsurvivors (Fig 5B). In the Cox regression multivariable analyses, Pro-C3 levels at stable state were associated with a shorter time to next exacerbation (hazard ratio, 0.72 [0.59-0.89]; $P = .002$), and Pro-C6 levels at stable state were associated with mortality (hazard ratio, 2.09 [1.18-3.71]; $P = .011$).

To demonstrate the potential predictive value of Pro-C6 that was found to be the best predictor of survival, a receiver operating-characteristic curve analysis was performed. As shown in Figure 6, survival of patients up

TABLE 3] Serum Levels of Extracellular Matrix Biomarkers and Their Association With the GOLD Grades in the PROMISE-COPD Cohort

Biomarker	PROMISE-COPD (N = 506)			
	GOLD II (n = 251)	GOLD III (n = 179)	GOLD IV (n = 76)	*P* Value
C3M	27.1 (21.3-33.4)	30.0 (24.4-35.1)	28.0 (21.8-34.9)	.010
C6M	12.6 (9.2-17.0)	14.1 (11.0-18.4)	12.9 (10.3-17.9)	.016
Pro-C3	11.2 (9.4-15.3)	10.4 (7.8-13.8)	10.2 (7.7-13.2)	.005
Pro-C6	8.0 (6.7-10.1)	8.1 (6.3-9.7)	7.5 (6.4-9.5)	.375
EL-NE	4.8 (1.6-9.4)	5.8 (1.6-10.3)	5.2 (1.6-9.4)	.257
Pro-C3/C3M	0.4 (0.3-0.6)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	< .001
Pro-C6/C6M	0.6 (0.5-0.9)	0.5 (0.4-.8)	0.6 (0.5-0.7)	.001

Values represent median (interquartile range [25th-75th percentile]) and are presented as nanograms per milliliter. C3M = fragments of collagen type III; C6M = fragments of collagen type VI; EL-NE = neutrophil elastase-generated fragments of elastin; Pro-C3 = pro-forms of collagen type III; Pro-C6 = pro-forms of collagen type VI. See Table 1 legend for expansion of other abbreviations.

to 2 years differed significantly between patients with Pro-C6 at stable state above or below the median for both the crude analysis ($P = .0241$) and the adjusted analysis ($P = .028$).

Discussion

We have previously shown that ECM turnover plays a major role in the lung function loss associated with recurrent exacerbations.[13-15] In the present study, we used recently developed assays to assess the levels of circulating neoepitopes for the systematic evaluation of the turnover of collagen type III, collagen type VI and elastin (molecules that have a paramount function in maintaining lung architecture) in two independent COPD cohorts. We showed that alterations in collagen and elastin metabolism were associated with lung function impairment (airflow limitation, hyperinflation, air trapping, and emphysema), disease severity (dyspnea, exercise capacity, and health-related quality of life), and bacterial colonization in stable COPD. The longitudinal assessment of circulating ECM markers at three points in time strongly suggests an increase in the activity of proteolytic enzymes at exacerbation, followed by a subsequent decrease to pre-exacerbation levels after 4 weeks. These neoepitopes, most likely generated by MMPs, were also found to be predictive for recurrence of exacerbation and survival in COPD. The associations with exacerbation and outcome proved to be independent of age, sex, extent of dyspnea, grade of airflow limitation, and comorbidities. To our knowledge, this study is the largest showing that ECM turnover, as assessed by using circulating neoepitope levels, is associated with disease severity, exacerbations, and survival in COPD.

All measured circulating markers of ECM turnover were associated with airflow limitations in the present study. Although degradation fragments of collagen type III and collagen type VI increased in patients with moderate to severe COPD, formation markers of collagen type III and collagen type VI presented the opposite behavior. These results suggest that there is an increase in turnover of collagen type III and collagen type VI parallel to an increase in COPD severity, as assessed according to lung function. The association between lung function and C3M, C6M, Pro-C6, and EL-NE was

TABLE 4] Association of Serum Levels of Extracellular Matrix Biomarkers With Lung Function Parameters in the PROMISE-COPD Cohort

Biomarker	PROMISE-COPD (N = 506)			
	FEV_1 % Predicted	*P* Value	FEV_1/FVC	*P* Value
C3M	0.998 (0.996-0.999)	.010	0.999 (0.996-1.001)	.290
C6M	0.995 (0.992-0.998)	.003	0.998 (0.994-1.001)	.180
Pro-C3	1.007 (1.004-1.009)	<.001	1.007 (1.004-1.010)	<.001
Pro-C6	1.003 (1.001-1.005)	<.001	1.004 (1.001-1.006)	.002
EL-NE	0.994 (0.990-0.999)	.030	0.998 (0.992-1.004)	.53

Data are presented as geometric mean ratios. See Table 1 and 3 legends for expansion of other abbreviations.

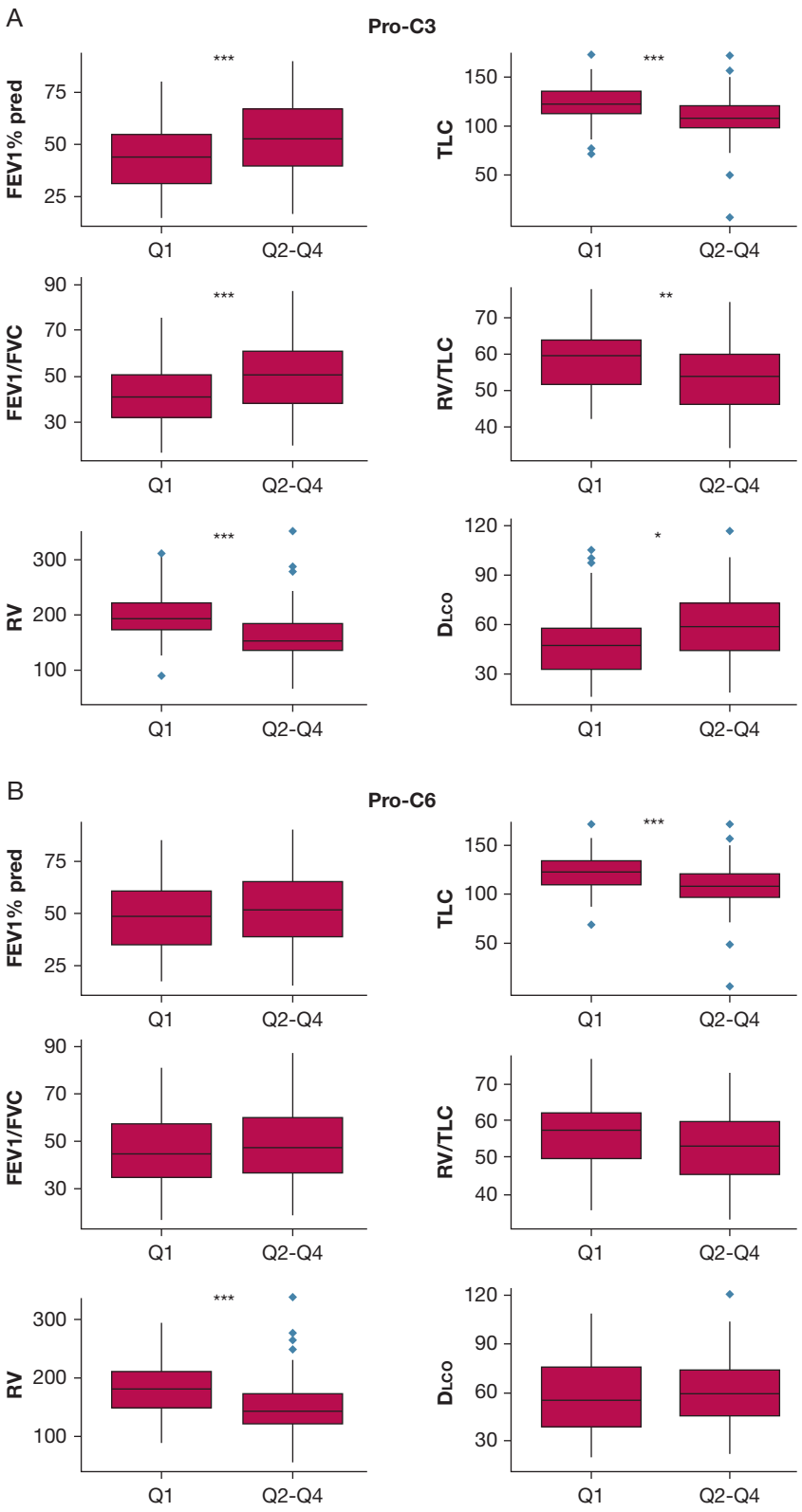

Figure 2 – *Lung function parameters according to the* distribution *of (A) pro-forms of collagen type III and (B) pro-forms of collagen type VI in quartiles (lowest Q_1 vs $Q_{2\text{-}4}$), in patients at stable state (N = 506) from the PROMISE-COPD cohort. Diamonds depict outliers.* ***P < .001, **P < .01, *P < .05. *See Figure 1 legend for expansion of abbreviation.*

not observed in any previous study, probably due to the small sample size.[18] Although elevated degradation markers of collagen type III and collagen type VI have been previously described in a small cohort of patients with mild COPD,[19] the association of degradation and formation of collagen type III and collagen type VI with airflow limitation severity (as assessed according to GOLD classification) is a new finding.

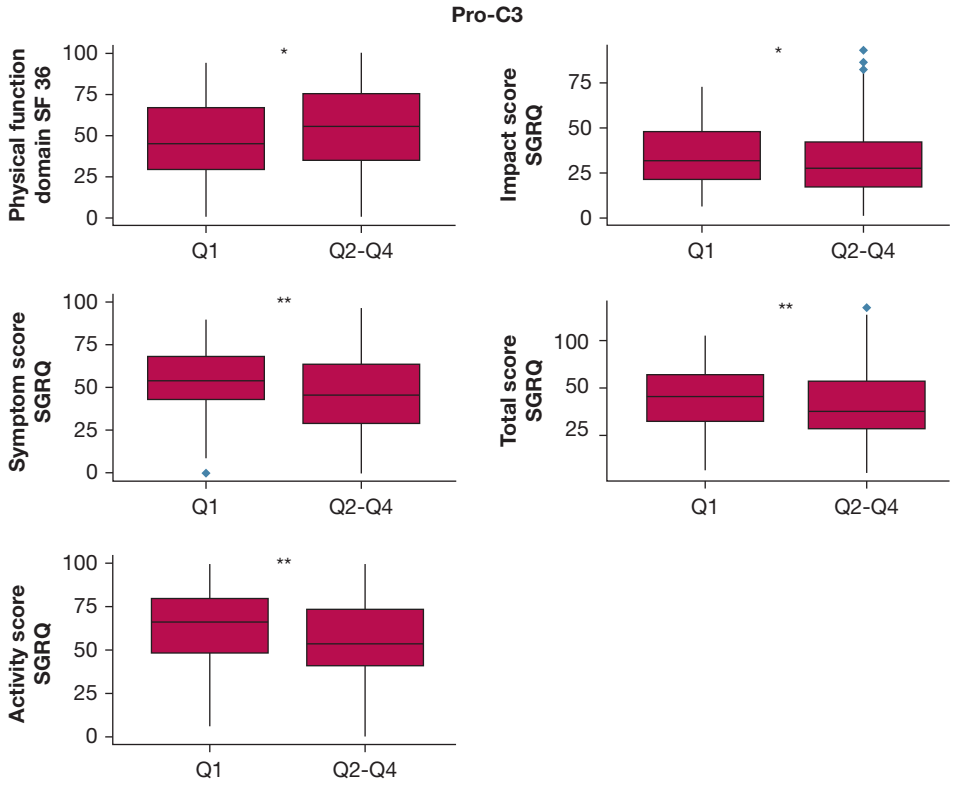

Figure 3 – *Association of pro-forms of collagen type III with health-related quality of life (SGRQ) and physical function domain of the SF-36, in quartiles (lowest Q_1 vs Q_{2-4}), in patients at stable state (N = 506) from the PROMISE-COPD cohort. Diamonds depict outliers.* **P < .01, *P < .05. *SF-36 = 36-item Short-Form Health Survey; SGRQ = St. George's Respiratory Questionnaire. See Figure 1 legend for expansion of other abbreviation.*

Collagen type III is an abundant ECM protein in the lung, and it has a paramount function in maintaining lung architecture. Furthermore, collagen type VI fibrils, together with other collagens and fibronectin, support tissue structure by adhering to the interstitial matrix. Thus, the interstitial matrix turnover seems to increase in parallel to disease severity in COPD. Interestingly, patients with the lowest levels of formation fragments of collagen type III and collagen type VI had more hyperinflation, air trapping, and emphysema. In our view, these differences, particularly for the diffusion capacity and residual volume, are of clinical relevance. This is the first time that systemic markers of degradation and formation of collagen type III and collagen type VI were associated with parameters obtained by using body plethysmography in COPD. Our results indicate that a deficient formation of collagen type III and collagen type VI may result predominantly in enhanced proteolysis in the stroma of pulmonary tissue.

TABLE 5] Health-related Quality of Life and Physical Function According to Pro-C3 Presented in Quartiles (Q_1 vs Q_2-Q_4) in the PROMISE-COPD Cohort (N = 506)

Variable	Pro-C3	*P* Value
Physical function domain SF-36	Q_1: 45.0 (28.8-66.2)	.019
	Q_{2-4}: 55.0 (35.0-75.0)	
Symptom score	Q_1: 54.3 (43.3-68.6)	.004
SGRQ	Q_{2-4}: 45.8 (28.6-64.4)	
Activity score	Q_1:66.0 (47.7-79.1)	.007
SGRQ	Q_{2-4}: 53.6 (41-72.8)	
Impact score	Q_1: 31.6 (20.9-47.5)	.044
SGRQ	Q_{2-4}: 27.5 (16.6-42.5)	
Total score	Q_1: 45.1 (32.0-57.2)	.009
SGRQ	Q_{2-4}: 37.4 (28.1-53.5)	

See Table 1 and 3 legends for expansion of other abbreviations.

We also found that C3M, C6M, and EL-NE levels were associated with the MMRC score. This finding disagrees with results of a previous report.[18] Taken together with the more severe impairment in the health-related quality of life observed in patients with increased collagen type VI degradation, this result seems plausible. Similarly, patients with decreased formation of collagen type III had the poorest health-related quality of life and the worst physical function, findings that match with the more severe impairment in lung function. This is the first time that health-related

TABLE 6] Serum Levels of Extracellular Matrix Biomarkers in Control Subjects and in Patients With COPD at Stable State Included in the BASCO-COPD Cohort

Biomarker	Control Subjects (n = 56)	BASCO-COPD Cohort (n = 100)	*P* Value
C3M	12.70 (10.60-15.30)	20.90 (17.10-25.30)	<.001
C6M	4.00 (4.00-4.00)	10.20 (4.00-17.40)	<.001
Pro-C3	10.50 (8.13-12.80)	11.30 (8.40-17.60)	.063
Pro-C6	7.31 (6.09-8.70)	9.68 (7.33-12.3)	<.001
EL-NE	1.87 (1.87-1.87)	1.87 (1.87-6.19)	<.001
Pro-C3/C3M	0.83 (0.54-1.32)	0.57 (0.41-0.81)	<.001
Pro-C6/C6M	1.71 (1.15-2.14)	1.14 (0.60-2.00)	.006

Data are presented as nanograms per milliliter. BASCO = Basel Study on COPD. See Table 1 and 3 legends for expansion of other abbreviations.

quality of life has been associated with ECM turnover as assessed systemically. We observed that bacterial colonization was associated with increased degradation of collagen type III, collagen type VI, and elastin. This finding supports the notion that bacterial colonization and high neutrophil count in sputum are factors that accelerate lung function decline in COPD.[20,21]

Our results showed increased degradation of collagen type III, collagen type VI, and elastin; increased

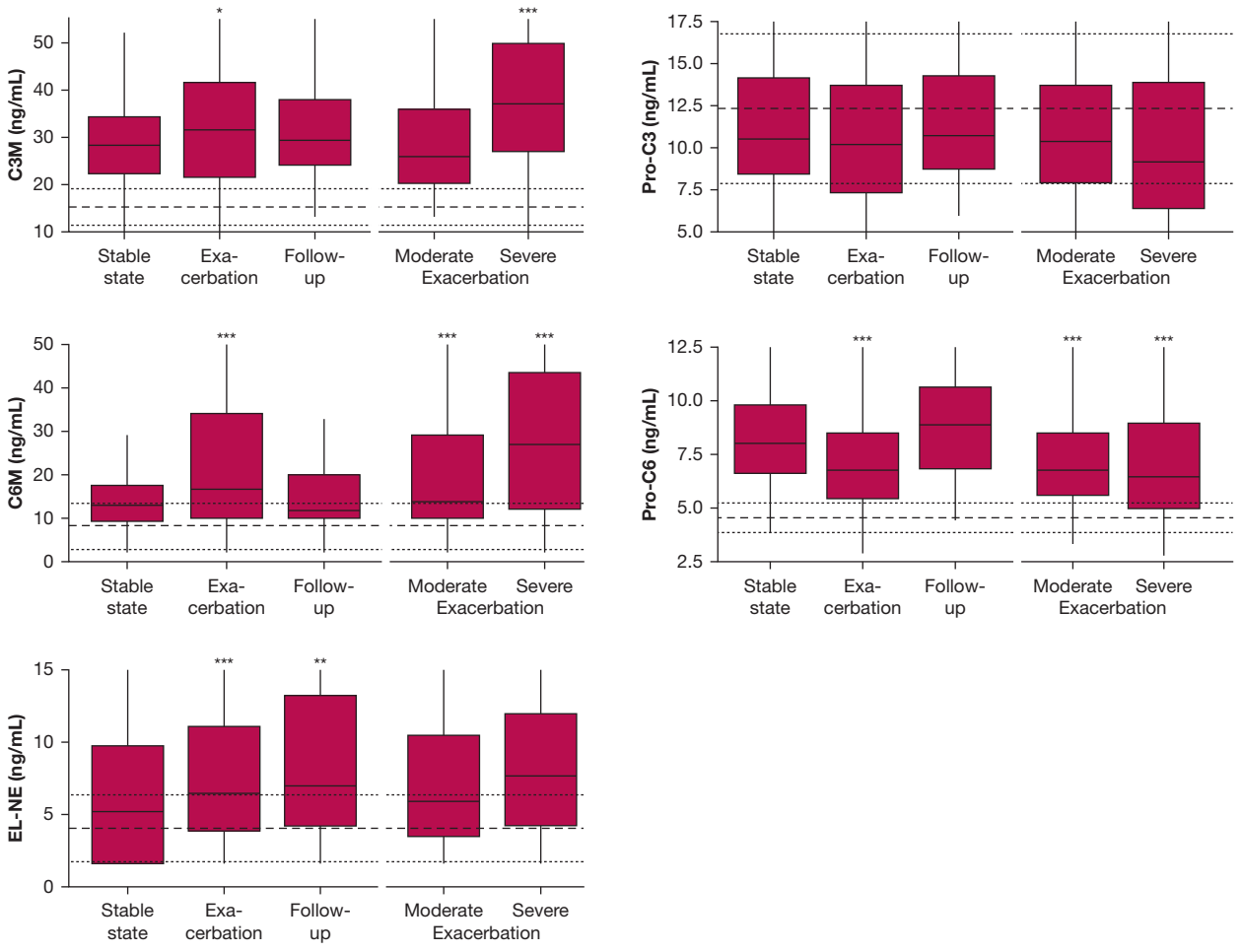

Figure 4 – *Serum levels of C3M, Pro-C3, C6M, Pro-C6, and EL-NE at stable state (N = 506), at exacerbation both moderate (n = 234) and severe (n = 115), and at follow-up of exacerbation (n = 104) adjusted for the clinical model. ***P < .001, **P < .01, *P < .05. C3M = fragments of collagen type III; C6M = fragments of collagen type VI; EL-NE = neutrophil elastase-generated fragments of elastin; Pro-C3 = pro-forms of collagen type III; Pro-C6 = pro-forms of collagen type VI.*

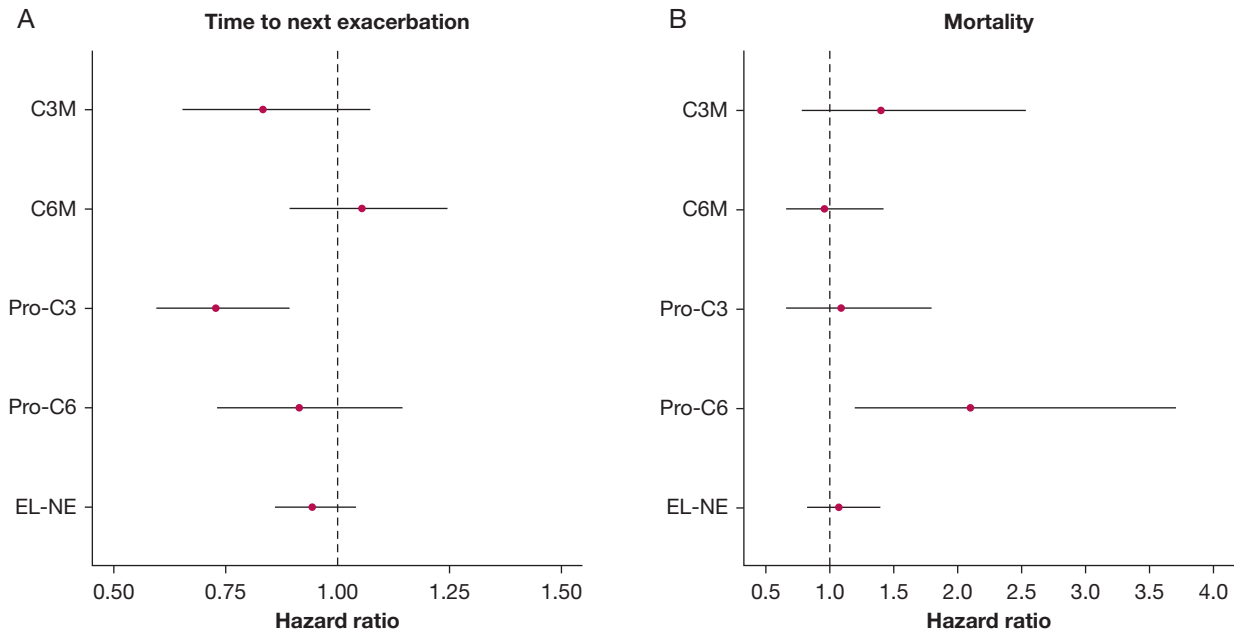

Figure 5 – *A, B, Risk of (A) exacerbation and (B) death for extracellular matrix biomarkers as estimated by Cox regression analyses after adjustment for the clinical model, in patients at stable state (N = 506) from the PROMISE-COPD cohort. See Figure 1 and 4 legends for expansion of other abbreviations.*

formation of collagen type VI; and no change in collagen type III formation at exacerbation. Due to the large sample size and comprehensive clinical characterization, we could also establish that these differences remained unaffected after adjustment in the clinical model. We found that degradation and formation of collagen type VI and EL-NE were increased both in moderate and severe exacerbations, whereas degradation of collagen type III was only increased in severe exacerbations. In contrast to previous data,[19] we found a difference in degradation and formation ratio for collagen type VI but not for collagen type III at exacerbation.

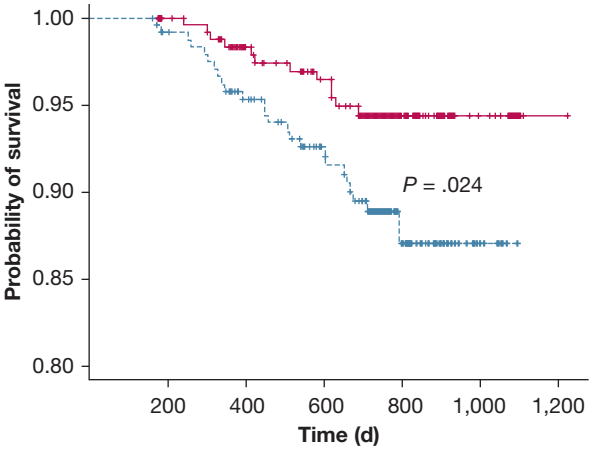

Figure 6 – *Receiver-operating characteristic curve analysis for pro-forms of collagen type VI, the best predictor for survival, dichotomized at the median at stable state.* P = *.0241 for the crude analysis and* P = *.028 for the analysis adjusted for the clinical model.*

Most extracellular collagens are initially degraded by MMPs (including MMP-1, -3, -7, -8, -13, -14, -16, and -18) that generate collagen fragments, which are further digested by MMP-2 and MMP-9.[22] Neoepitope fragments of collagen type II and collagen type III generated by MMPs have been shown to reflect disease activity in rheumatoid arthritis and to predict response to anti-IL-6 treatment with tocilizumab.[23] Similarly, related markers such as MMP-generated neoepitopes of collagen type I and collagen type VI have been discussed as appropriate markers for disease activity in idiopathic pulmonary fibrosis.[24]

It remains to be clarified if the increased levels of C3M and C6M that we report in the present study reflect increased serum levels or increased activity of MMPs. In this context, these results agree with the increased levels of MMP-9, TIMP-1, TIMP-2, heparan sulfate, chondroitin sulfate, and hyaluronic acid in the BAL at exacerbation that we have previously described.[13-15]

An additional new finding is that, except for EL-NE, levels of C3M, C6M, and Pro-C6 returned to basal levels measured at stable state within 4 weeks after exacerbation. These results indicate that the ECM turnover associated with exacerbations in COPD was an acute event and that there are repair mechanisms which contribute to balancing the increased degradation of ECM within 4 weeks following exacerbations of COPD. We must still determine if the structural changes that

result from the ECM turnover remain in the stroma of pulmonary tissue after each exacerbation. The elevated levels of EL-NE that persist 4 weeks after exacerbation support the hypothesis that each exacerbation may initiate a degradation process of ECM molecules that, in addition to chronic remodeling, may lead to accumulating structural and functional changes of the lung ECM, increased emphysema, and lung function limitation that accumulate with time. This outcome may also explain why patients with recurrent exacerbations have an accelerated decline in lung function.

We believe these findings are important for the following reasons: (1) they demonstrate in a large, multicentric cohort that MMP- or NE-derived collagen and elastin turnover is increased at exacerbation in moderate to very severe COPD; (2) they strongly suggest that differences in ECM molecules at stable state and exacerbation are independent of age, sex, dyspnea, baseline lung function impairment, and the presence of comorbidities; (3) they indicate that exacerbations affect the ECM in a heterogeneous way (ie, although the degradation and formation of collagen type VI are increased, there is no change in collagen type III); (4) they infer that ECM neoepitopes could be used to identify the incidence of exacerbation and, potentially, its severity; and (5) they propose that the lung function decline subsequent to exacerbation could be induced, and thus potentially handled, by interventions directed toward ECM turnover at exacerbation. Thus, measurement of serum neoepitopes would allow better understanding of COPD pathogenesis and may enable a more focused and shorter proof-of-concept trial with fewer patients, thus assisting in innovative drug development.[25]

We evidenced new associations of ECM neoepitopes at stable state and clinical outcome up to 2 years in COPD: formation fragments of collagen type III were increased in those at higher risk of exacerbation, whereas both formation of collagen type VI and its balance at stable state were predictive of mortality. An increase in collagen degradation biomarkers was also shown to be associated with disease progression, and the rate of this increase predicted survival in patients with idiopathic pulmonary fibrosis,[17] a progressive interstitial lung disease characterized by ECM remodeling.[26,27] The results presented here suggest that protein fragments might provide an indirect measure of disease activity, particularly dynamic remodeling, of lung tissue also in COPD. Common to both diseases is the highly altered interaction between fibrogenesis and fibrinolysis leading to functional impairment of the lungs.[19] Therefore, it may be postulated that similar mechanisms of collagen degradation and formation leading to lung tissue remodeling may be shared between idiopathic pulmonary fibrosis and COPD. However, there is an urgent need to confirm these findings because they have the potential to improve and shorten the assessment of new treatment in COPD.

This study has a few limitations. We assessed circulating ECM turnover markers at stable state in two independent cohorts but did not evaluate dynamic changes of biomarkers in patients with stable disease longitudinally. Thus, although we could show an association of collagen and elastin biomarkers with exacerbation and outcome in COPD, we cannot exclude that the rate of change of these neoepitopes could have been even more informative to predict disease worsening and reduced survival. Further longitudinal studies are needed to elucidate if these neoepitopes are the cause or the result of disease pathogenesis.

Conclusions

Serologic biomarkers of ECM turnover were significantly associated with disease severity and clinically relevant outcomes in patients with COPD.

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Additional information: The e-Appendix and e-Tables can be found in the Supplemental Materials section of the online article.

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