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Collagen Degradation and Formation Are Elevated in Exacerbated COPD Compared With Stable Disease

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KEYWORDS: basement membrane; C1; C4; cell turnover; collagen; COPD; ECM; lamina reticularis; type I collagen; type IV collagen

ABSTRACT

BACKGROUND: The role of the extracellular matrix (ECM) structure and remodeling thereof in lung diseases is gaining importance. Pathology-related changes in ECM turnover may result in deleterious changes in lung architecture, leading to disease in the small airways. Here, degradation fragments of type I (C1M), type IV (αl chain, C4M2), and type IV (α3 chain, C4Ma3) collagen, all degraded by metalloproteinases and the pro-form of collagen type V (PRO-C5) were investigated and associated with COPD severity and outcome.

METHODS: In a prospective, observational, multicenter study including 498 patients with COPD Gold Initiative for Chronic Obstructive Lung Disease stage 2 to 4, ECM markers were assessed in serum at stable state, exacerbation, and at follow-up 4 weeks after exacerbation.

RESULTS: At stable state, there was a significant inverse association between FEV1 % predicted and C1M, C4Ma3, and Pro-C5. C1M, C4M2, C4Ma3, and Pro-C5 were associated with BMI, airflow obstruction, dyspnea, and exercise capacity (BODE) index and the modified Medical Research Council (MMRC) score. C1M, C4M2, C4Ma3, and Pro-C5 were significantly increased from stable

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state to exacerbation and decreased at follow-up. Furthermore, the biomarkers were significantly higher during severe exacerbation compared with moderate exacerbation. Multivariate analysis adjusted for BMI, MMRC score, unadjusted Charlson score, and FEV1 %predicted showed a significant influence of C1M, C4Ma3, and C4M2 on time to exacerbation. None of the biomarkers were predictors for mortality.

CONCLUSIONS: Serologically assessed collagen remodeling appears to play a significant role in COPD severity (airflow limitation, dyspnea) and disease outcome (time to exacerbation and prognosis as assessed by the BODE index).

ABBREVIATIONS: 6MWT = 6-min walk test; ADM = adrenomedullin; BODE = BMI, airflow obstruction, dyspnea, and exercise capacity; C1M = type I collagen; C4M2 = type IV alpha-1 chain; C4Ma3 = type IV alpha-3 chain; ECM = extracellular matrix; GOLD = Gold Initiative for Chronic Obstructive Lung Disease; MMP = metalloproteinase; MMRC = modified Medical Research Council; Pro-C5 = type V collagen pro-form

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COPD is characterized by various conditions that result in airflow limitation and an abnormal inflammatory response of the lung.[1] Inflammation contributes to airflow limitation by causing excessive remodeling of the airway wall.[2] Lung extracellular matrix consists of cartilage, provisional matrix, a basement membrane, and interstitium.[3] The basement membrane is derived mainly from type IV collagen, which forms the most abundant nonfibrillar collagen in the lung.[3] The alpha-3 chain of type IV collagen is specific to lung and kidney tissue. It plays an important role in cellular proliferation, adhesion, migration, and differentiation. Collagen types I and V are fibrillar collagens. Fibrillar collagens allow the lung to maintain its shape during inflation and deflation and form 15% to 20% of the dry weight of the lung.[3] Collagen types I and V are two of the collagens forming the lamina reticularis, which is a thin layer below the basement membrane.[4,5] Thickening of the lamina reticularis leads to decreased airway distensibility and thus increased airway limitation.[6] Collagen type V is an autoantigen that plays a role in lung transplant rejection and has been shown to correlate to fibrosis. Immunotherapy with collagen type V in patients with interstitial pulmonary fibrosis resulted in stabilized FVC[7]; in animals, it resulted in a reduction in lung inflammation and lung fibrosis.[8]

We and others have previously demonstrated that collagen degradation is up-regulated in patients with COPD[9,10] and is associated with some clinically relevant outcomes of COPD.[11"13] Sand et al[9] showed that the degradation fragments of collagen types III, IV, and VI are increased during exacerbation of COPD compared with follow-up after the exacerbation, but found no association between these factors and disease severity. Degradation fragments of collagen type I and the formation fragments of collagen type VI are inversely associated with FEV_1.[14] Collagen type I degradation is associated with increased mortality in patients with interstitial pulmonary fibrosis[15] and in patients with COPD.[12] Thus far, no data exist regarding the longitudinal expression of the degradation fragments of collagen types I and IV and the formation fragment of collagen type V in COPD stable state, exacerbation, and follow-up. We hypothesize that these particular collagens are associated with disease severity and outcome in COPD.

Methods

STUDY DESIGN AND PATIENTS

Patients in stable state COPD with Gold Initiative for Chronic Obstructive Lung Disease (GOLD) 2 to 4 were enrolled in the Predicting Outcome Using Systemic Markers in Severe Exacerbations of Chronic Obstructive Pulmonary Disease multicenter trial; an observational prospective trial performed in 11 centers in 8 European countries. The study details have been published previously.[11] The study was investigator-initiated and driven and carried out according to the Declaration of Helsinki and Good Clinical Practice guidelines. The institutional review board approved the study (EKBB295/07) and it was registered at www.controlled-trials.com (identifier ISRCTN99586989).

Patients were followed for at least 2 years at scheduled half-yearly visits. All participating patients had a baseline examination at stable state and were monitored for recurrent moderate (requiring

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treatment with systemic corticosteroids, antibiotics, or both) and severe (requiring hospitalization or a visit to the ED) exacerbations. A follow-up visit was performed 4 weeks after the onset of exacerbation. Clinical history, physical examination, lung function, and the 6-min walk test (6MWT) were performed, and the patients completed the Modified Medical Research Council Score (MMRC), the St. George's respiratory questionnaire COPD version, and the 36-item Short-Form health survey. The latter is a health-related quality of life questionnaire. The evaluation of biomarkers associated with the outcome of the disease was a predetermined end point of the study.

DETERMINATION OF BIOMARKERS

Serum levels of fragments of collagen type I (C1M), collagen type 4 (αl chain C4M2 and α3 chain C4Ma3), and the pro-form of collagen type V (Pro-C5) were measured with Nordic Bioscience assays according to the manufacturer's instructions.[11,15] All assays used a competitive setup with monoclonal antibodies directed against either a protein fragment produced by the metalloprotease cleavage during degradation or formation, or an internal protein sequence as described previously.[15] This means that the assay assesses collagen fragments in which either the N- or C-terminal end is known (depending on the assay); therefore, not only one molecular size is measured, because the length varies depending on how far upstream or downstream from this site it goes. Various fragment lengths were formed depending on how far up- or downstream from the N- or C-terminal was measured.[10,16] A western blot would most likely show a smear of different lengths detected by the antibody. This is a strength of the assay because the assay is not limited to detecting only one fragment, which may be undetectable because of low concentrations. The choice of the fragments to be assessed was made on the basis of previously investigated pathophysiological processes in COPD or data-driven for the pioneer discoveries. All samples were analyzed in duplicate by personnel blinded to patients' clinical data.

STATISTICS

Differences in dichotomous variables were evaluated using the χ^2 test or Fisher exact test, as appropriate. Normally distributed parameters were analyzed using the Student *t* test for equality of means. All other continuously non-normally distributed parameters were evaluated using the nonparametric Mann-Whitney *U* test or Kruskal-Wallis test, as appropriate.

The association between the ECM biomarkers and COPD clinical parameters was evaluated by univariate linear regression models. Analyses were conducted both unadjusted and adjusted for the clinical characteristics such as age, BMI, FEV_1 % predicted, MMRC, and unadjusted Charlson comorbidity index score 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.

The Statistical Package for Social Sciences Program (SSPS Inc, version 22, for Windows) was used. All tests are two-tailed; a P value $< .05$ was considered significant. Results are expressed as mean (SD) or median (interquartile range), unless otherwise stated.

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Results

A total of 498 patients were included in the study (Fig 1). The majority of the participants were men (72%) and the median age was 67 years (Table 1).

All collagen biomarkers increased from stable state to exacerbation and then returned to baseline levels at follow-up 4 weeks after the exacerbation (Fig 2). C1M median 24.3 vs 41.2 vs 25.8 ng/mL, C4M2 median was 54.6 vs 65.5 vs 60.2 ng/mL, C4Ma3 median was 5.7 vs 7.2 vs 5.9 ng/mL, and Pro-C5 median was 469.0 vs 560.1 vs 476.9 ng/mL.

There were significantly higher levels of the collagen remodeling biomarkers during severe exacerbation compared with moderate exacerbation (Fig 3). C1M median was 70.9 vs 31.4 ng/mL, C4M2 median was 75.6 vs 61.0 ng/mL, C4Ma3 median was 9.3 vs 6.2 ng/mL, and Pro-C5 median 643.2 vs 450.2 ng/mL.

COLLAGEN BIOMARKERS AND LUNG FUNCTION

C1M, C4Ma3, and Pro-C5 were significantly associated to FEV1 % predicted (Table 2) also when adjusting for number of severe exacerbations, BMI, smoking status, and unadjusted Charlson score. None of the collagen biomarkers were associated with FVC % predicted. All the investigated collagen biomarkers showed a significant association with FEV1/FVC % (Table 2).

There were significant changes in C1M (median, 22.3 ng/mL, 25.3 ng/mL, 27 ng/mL; P = .021); C4M2 (median, 53.0 ng/mL, 55.5 ng/mL, 58.6 ng/mL; P = .018); C4Ma3 (median, 5.2 ng/mL, 6.0 ng/mL, 5.8 ng/mL P = .002), and Pro-C5 (median, 448.6 ng/mL, 488.9 ng/ mL, 474.7 ng/mL; P = .009), dependent on the GOLD status (GOLD 2 to 4, respectively) of the patient.

COLLAGEN BIOMARKERS AND DISEASE EFFECT

Only C1M was significantly associated with the unadjusted Charlson score (r = 0.106; P = .032), but all the biomarkers were highly significantly associated with BMI, airflow obstruction, dyspnea, and exercise capacity (BODE) index (Table 3). Both C4Ma3 and Pro-C5 positively associated with pack years and in turn negatively associated with the 6MWT. All biomarkers were positively associated with MMRC, which represents the degree of dyspnea. There was no association between the biomarkers and the various parameters used to determine quality of life except between C1M and activity score (r = 0.100; P = .048) as measured using the St. George's respiratory questionnaire.

The biomarker levels did not differ significantly between smokers and nonsmokers. There were significant differences in C1M, C4Ma3, and Pro-C5 between patients taking and not taking inhaled corticosteroids (ICS) (Table 4). With multiple regression adjusting for the various variables that have shown an association with the biomarkers (Table 3), the association between the biomarkers and ICS disappeared.

None of the collagen biomarkers was associated with exacerbation rate or with severe exacerbation rate. Cox regression analysis showed that C4Ma3 and Pro-C5 significantly influenced time to exacerbation in the first year of follow-up (Fig 4). Lower levels of C4Ma3 (P = .05) and Pro-C5 (P = .02) resulted in less time to exacerbation in the first year of follow-up, but

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this effect disappeared after 2 years. Multivariate Cox regression adjusted for BMI, MMRC score, unadjusted Charlson score, and FEV1 % predicted, showed a significant influence of C1M (hazard ratio [HR], 1.008; P = .013), C4Ma3 (HR, 0.876; P = .007), and C4M2 (HR, 1.014; P = .039) on time to exacerbation in the 2 years of follow-up.

Figure 1 *- Study design. GOLD = Gold Initiative for Chronic Obstructive Lung Disease.*

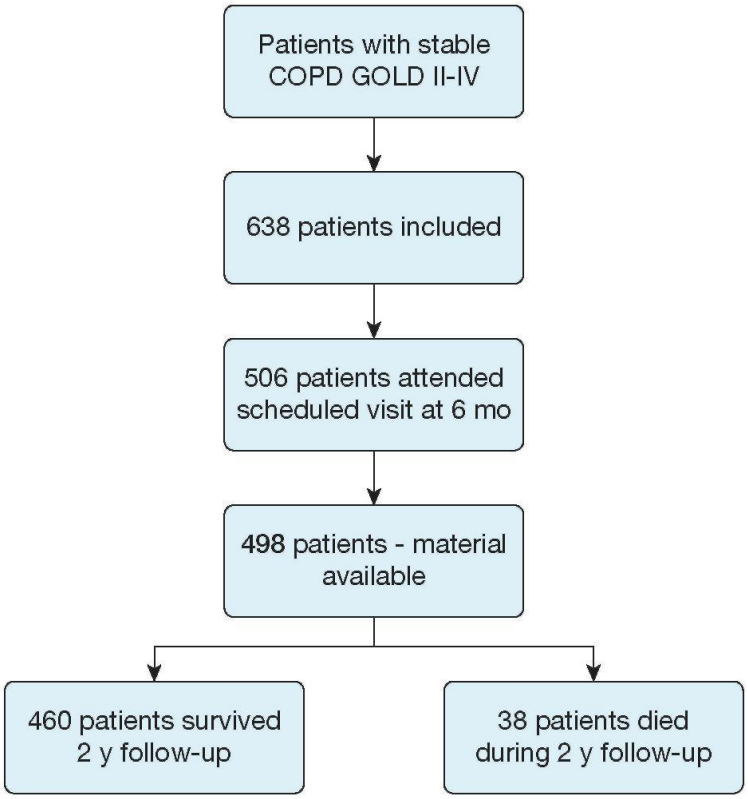

Table 1 *- Basic Characteristics of Patients in the Study*

Characteristic	Data
Men	358 (72)
Age, y	67 (13)
Current smoker	144 (29)
Pack y	45 (35)
BMI, kg/m^2	26 (6.2)
BODE index	2 (3)
6MWT, m	398 (120)
Exacerbation rate	1(0)
Severe exacerbationrate	0 (0)
Unadjusted Charlson Score	1(1)
MMRC score	2(1)

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Lung function (post-brd)	
FEV_1, L	1.3 (0.7)
FVC, L	2.7 (1.2)
FEV_1, % predicted	50 (27)
FVC, % predicted	80 (26)
GOLD status[3]	
II	248 (50)
III	168 (34)
IV	77 (16)
Inflammation markers (n = 518)	
Copeptin, pmol/L	8.5 (12.2)
Adrenomedullin, nmol/L	0.6 (0.3)
Atrial natriuretic peptide, pmol/L	83.8 (81.2)
Procalcitonin, ?g/L	0.08 (0.03)
SF-36	
Physical function	50 (45)
Role physical	50 (100)
Role emotional	100 (83)
Social functioning	75 (50)
Mental health	65 (25)
Body pain	80 (48)
Vitality	50 (31)
General health	50 (37)
SGRQ	
Symptoms score	49 (34)
Activity score	57 (32)
Impact score	29 (27)
Total score	39 (27)

Continuous data are presented as median (interquartile range) and categorical variables as No. (%). 6MWT = 6-min walk test; BODE = BMI, airflow obstruction, dyspnea, and exercise capacity; brd = bronchodilator; GOLD = Gold Initiative for Chronic Obstructive Lung Disease; MMRC = modified Medical Research Council; SF-36 = 36-item Short-Form Health Survey; SGRQ = St. George's Respiratory Questionnaire. [a]GOLD grades are on the basis of FEV_1 % predicted: 50% ≤ II ≤ 80%; 30% ≤ III ≤ 50%; and IV ≤ 30%.

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Discussion

Changes in the turnover of extracellular matrix (ECM) molecules in the lung result in changes in lung architecture that may play a role in lung disease. This is the first study to longitudinally investigate the role of collagen types I, IV, and V in COPD during stable state, exacerbation, and follow-up. We found that the degradation fragments of collagen types I and IV and the collagen V formation were increased during exacerbation and decreased to basal levels 4 weeks after exacerbation. These molecules were significantly higher during severe exacerbation compared with moderate exacerbation. All the collagen biomarkers except for C4M2 were significantly negatively associated with FEV_1 % predicted. In addition, there was a significant association with circulating adrenomedullin, a marker of poor outcome in COPD.

COPD is associated with changes in airway composition and structure characterized by small airway inflammation that results in epithelial damage, airway fibrosis, and remodeling of the ECM, affecting both interstitial matrix and basement membrane in the lung.[17-19] Whereas activated fibroblasts deposit ECM proteins, such as collagens I and III, pathological proteases including matrix metalloproteinases (MMPs) 1, 2, 7, 9, and 12 and elastase are overly expressed in COPD.[20-22] Of interest, FEV_1 values were previously found to be inversely correlated with collagens in the surface epithelial basement membrane in the lung, suggesting that increased bronchial deposition of collagen contributes to deteriorated lung function and airway remodeling in COPD.

The basement membrane, which consists of type IV collagens, functions in anchoring the epithelium to the connective tissue as a mechanical barrier and plays a role in angiogenesis. Destruction of the basement membrane as suggested by the increase in C4M2 fragments was associated with poorer lung function (decrease in FEV_1/ FVC), more severe disease (as measured by the BODE index), increased dyspnea (as measured by the MMRC score), and an increase in the inflammatory marker adrenomedullin (ADM). ADM[24] and collagen type IV[12] degradation have been associated with increased mortality in patients with COPD and with exacerbation rate. We found no association between collagen type IV degradation and mortality or exacerbation rate, but there was an increase in collagen type IV in severe exacerbation compared with moderate exacerbation.

The type IV alpha-3 subunit, although forming part of collagen type IV and thus part of the basement membrane, appears to have a phenotype more resembling

that of collagen type V than the type IV alpha-1 subunit, C4M2. C4Ma3 and Pro-C5 were associated with pack years, BODE index, 6MWT, MMRC score, copeptin, ADM, and atrial natriuretic peptide. Also, only C4Ma3 and Pro-C5 had a significant effect on time to exacerbation in the first year of follow-up. Of interest, no association between C4Ma3 and pack years, 6MWT, or MMRC score was found previously[9] The discrepancy in results could be because of the small population size in the former study[9] Not much more is known about C4Ma3 except that mutations in the gene encoding this protein result in a disease called Alport syndrome and some of the symptoms include dysphagia, recurrent bronchitis, dyspnea, cough, and stridor.[25]

Collagen types I and V are two of the collagens forming the lamina reticularis, which forms part of the reticular basement membrane.[4,5] Thickening of the lamina reticularis, as evidenced in

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asthma, leads to decreased airway distensibility and thus increased airflow limitation.[4,6,20] Increased ECM turnover, as suggested by the increase in collagen type I fragmentation, had a negative association with $FEV_{1;}$ similar to the results by Bihlet et al.[14] Unlike Dragsbaek et al[26] and Sand et al,[12] we found no association between collagen type I MMP degradation and mortality. Collagen type V is an autoantigen that plays a role in lung transplant rejection and has been shown to correlate to fibrosis. Immunotherapy with collagen type V in patients with interstitial pulmonary fibrosis resulted in stabilized FVC[7]; in animals, it resulted in a reduction in lung inflammation and lung fibrosis.[8] We found no association between increased collagen type V and FVC, but found a positive correlation with the inflammatory markers copeptin, ADM, and atrial natriuretic peptide.

The relationship between smoking status in COPD and collagen turnover is unclear. One would expect that smoking has some influence on the collagen biomarkers because smoking increases inflammation, which may drive some of the ECM remodeling through changes in protease expression and activity. In line with two previous studies,[9,14] we found no association between collagen biomarkers and current smoking status. Bihlet et al[14] show no significant difference in collagen biomarker levels in healthy smokers compared with healthy nonsmokers, nor in obstructive, nonemphysematous COPD, and healthy smokers or nonsmokers. Combining emphysematous and nonemphysematous COPD shows that collagen VI and elastin degraded by neutrophil elastase are significantly higher than in nonsmoking control patients. Collagen I has no association with smoking status. Of interest, in the complete COPD group, there are only five (9.6%) current smokers. In the smoking group, 12 (48%) are current smokers and the rest are ex-smokers. On the one hand, it is unclear whether the difference between the COPD group and the nonsmoking group remains if adjusted for other factors such as Charlson Score or $FEV_{1;}$ because it is possible that the difference is due to the patients having COPD and not from the effects of smoking status. That no difference was seen between healthy smokers and healthy nonsmokers gives credence to this assumption. On the other hand, in our study and the study of Sand et al,[9] most of the COPD nonsmokers were ex-smokers. It is possible that the effects of past smoking results in long-term changes in biomarker levels, which because of the occurrence of exacerbations, which also affect biomarker levels, are then indistinguishable from the increase caused by smoking currently. There is also the possibility that collagen biomarker levels are not only driven by inflammation because acute inflammation models in humans and rodents show no effect on the markers (J. M. B. Sands, unpublished data, 2018). More studies are required to elucidate the relationship between collagen markers and smoking status.

The severity of the airflow limitation, as assessed by the GOLD classification, was positively associated with the levels of the collagen markers. Patients with GOLD 4 had more ECM turnover than patients with GOLD 2. This was also evident in patients receiving ICS, who had significantly more ECM turnover than the patients not receiving ICS. Although counterintuitive, the increase in collagen biomarkers seen in ICS-treated patients is not surprising. Children with asthma taking ICS have increased glycosaminoglycans in their urine compared with those on relief medication.[27] Glycosaminoglycans are proteoglycans forming important structural and functional components of the ECM. Priftis et al[27] postulated that the increase in urinary glycosaminoglycans was due to ICS reversing the remodeling caused by asthma. In asthmatic adults, the long-term use of ICS does not decrease the reticular basement membrane thickness compared with patients having asthma but not using ICS; unfortunately, the circulating levels of

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collagen biomarkers were not measured.[28] In adult patients with COPD, a 6-month high-dose ICS treatment had no effect on hypervascularity or angiogenic growth factors in the reticular basement membrane.[29] Again, no circulating collagen biomarkers were measured. An in vitro study on human lung fibroblasts exposed to 5% fetal calf serum, which mimics the inflammatory milieu, showed that budesonide treatment increased ECM and collagen deposition.[30] Steroid treatment affects collagen levels. It is unclear whether the effect is to reverse the remodeling caused by asthma and COPD or whether the effect is to maintain the status quo and thus prevent further deterioration.

Inflammation contributes to airflow limitation by causing remodeling of the airway wall.[2] Collagen types I and IV degradation fragments and type V formation fragments were increased during exacerbation, which is characterized by an increase in inflammation. Similarly, it was previously shown that during exacerbations of COPD, serum, and urine MMP-9 levels were significantly elevated by 17% and 30% compared with recovery values.[23] There were relevant differences in MMP-8 levels and activity in the different matrices: although serum levels depicted unchanged values over a week in patients with stable disease, urine MMPs varied by up to ninefold in the same population.[23] Results of our study support the notion that the ECM derangement resulting in airway remodeling and progressive airflow obstruction at exacerbation can be systemically assessed by the determination of circulating fragments of collagen degradation and stand in line with a prior study demonstrating higher degradation of collagen I and VI in those with a greater loss in lung function over time.[31] It is tempting to hypothesize that a compound able to prevent collagen degradation, particularly at exacerbation, might beneficially influence the vicious cycle of recurrent exacerbations and airflow limitation. These markers were higher during severe exacerbation (ie, requiring hospitalization compared with mild/ moderate exacerbation). Although collagen types III, IV, and VI are increased during exacerbation compared with the follow-up after the exacerbation, this is the first study to investigate whether there are differences in collagen biomarkers between severe and mild/moderate exacerbations.

This study has several limitations, including the fact that we did not measure changes in ECM markers in stable COPD longitudinally.

Figure 2 *- A, C1M, B, C4M2, C, C4Ma3, and D, Pro-C5 during stable phase (n = 438), exacerbation (n = 327), and at follow-up 4 weeks after exacerbation (n = 109). C1M (P < .001), C4M2 (P < 001), C4Ma3 (P < 001), and Pro-C5 (P < 001) significantly increased from baseline to exacerbation and then decreased back to baseline 4 weeks after the exacerbation (follow-up). P <05 was considered significant. C1M = Type I collagen; C4M2 = Type IV alpha-1 chain; C4Ma3 = Type IV alpha-3 chain; Pro-C5 = Type V collagen pro-form.*

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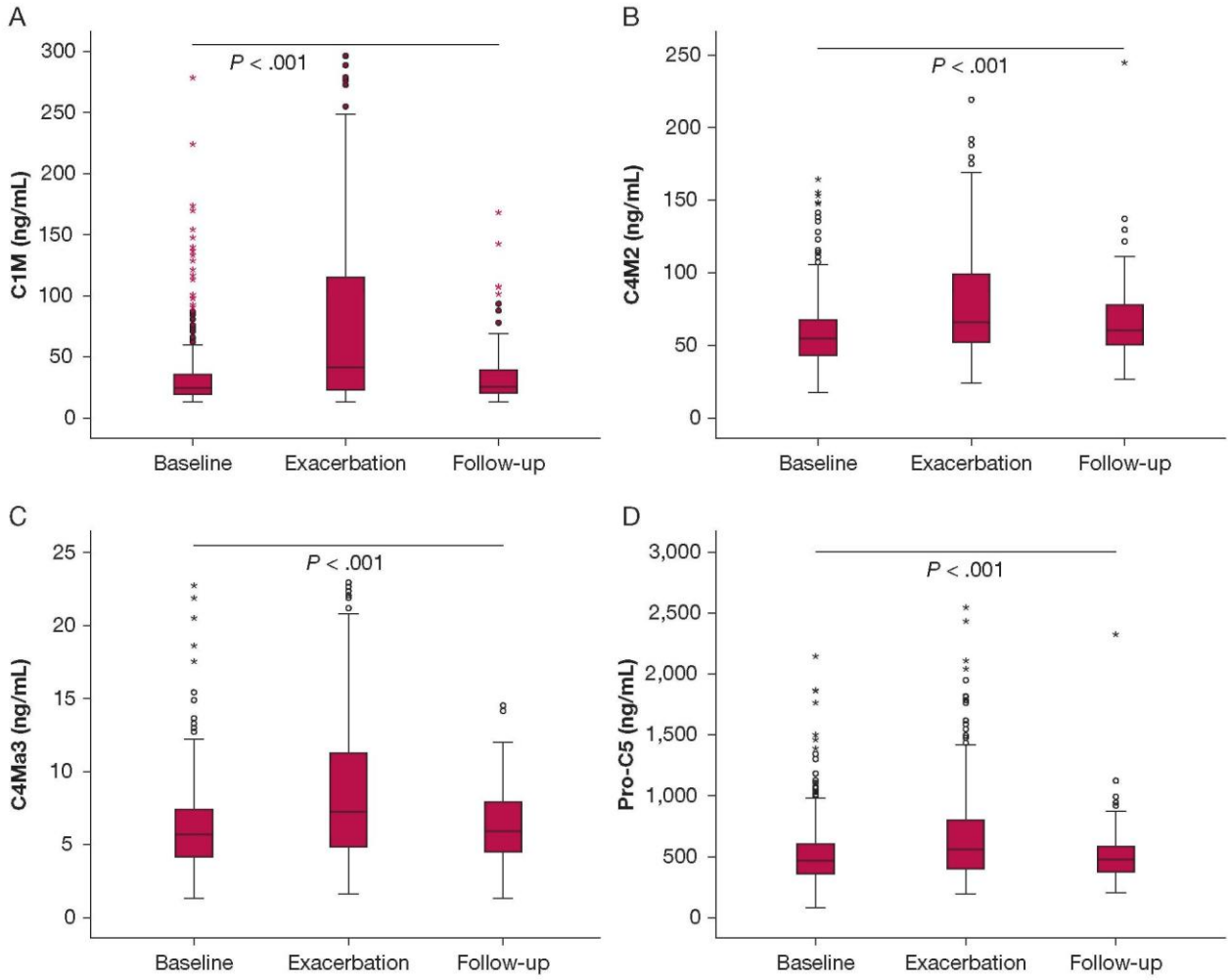

Figure 3 *- Boxplot depicting significantly more A, C1M (P < .001); B, C4M2 (P = .040); C, C4Ma3 (P = .001), and D, Pro-C5 (P < .001) during severe exacerbation (n = 114) than during moderate exacerbation (n = 154). P < .05 was considered statistically significant. See Figure 2 legend for expansion of abbreviations.*

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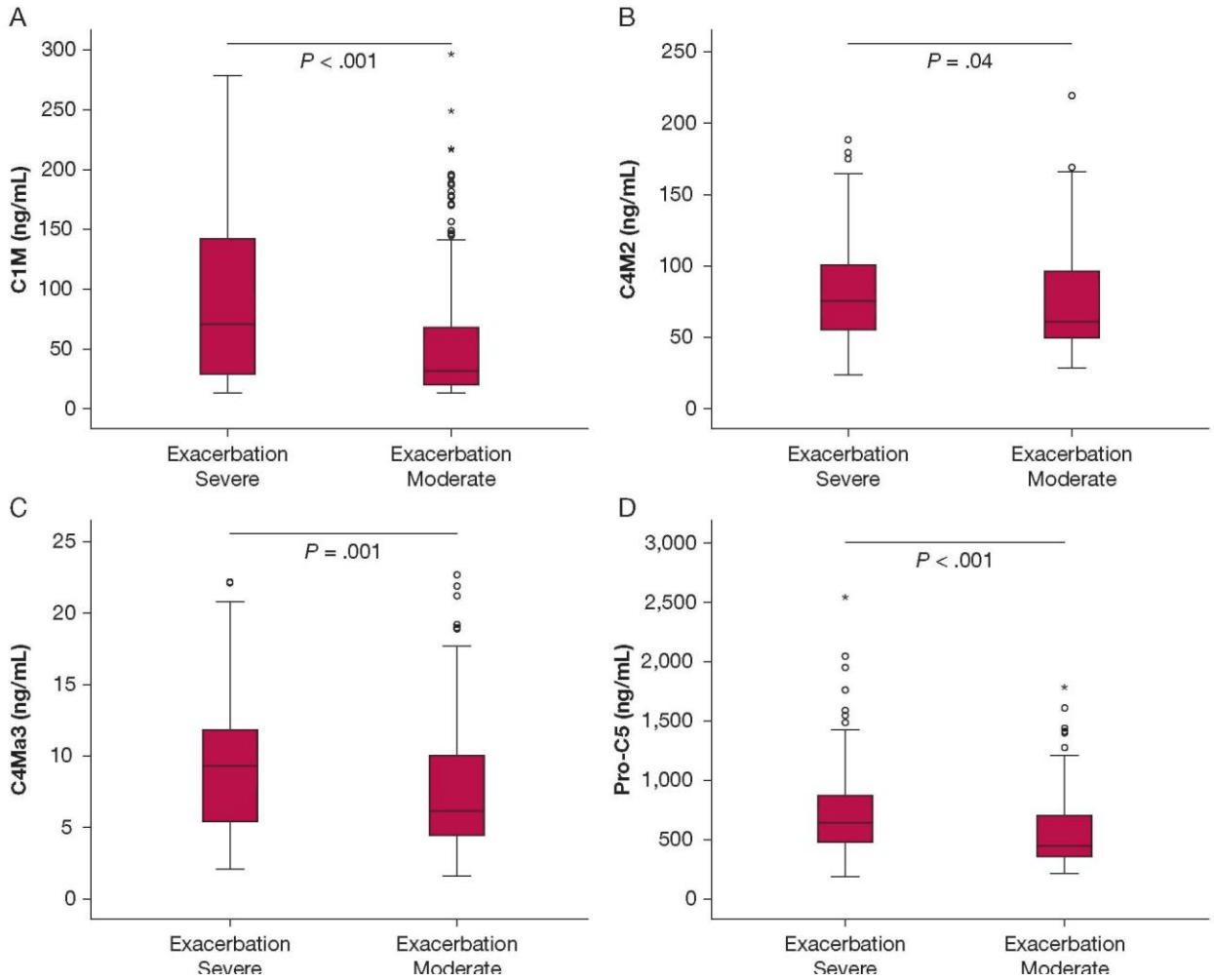

Table 2 *- Univariate Linear Regression of the Association Between Lung Function (Postbronchodilator) and the Different Collagens*

Biomarker	Beta	FEV_1, % Predicted	Beta	FVC, % Predicted	Beta	FEV_1/FVC, %
C1M	**-0.152**	**0.003**	-0.038	0.468	**-0.154**	**0.002**
C4M2	-0.100	0.052	0.022	0.675	**-0.144**	**0.004**
C4Ma3	**-0.146**	**0.005**	-0.096	0.063	**-0.113**	**0.026**
Pro-C5	**-0.139**	**0.006**	-0.056	0.271	**-0.123**	**0.014**

Boldface type highlights the statistically significant associations. C1M = Type I collagen; C4M2 = Type IV alpha-1 chain; C4Ma3 = Type IV alpha-3 chain; Pro-C5 = Type V collagen pro-form.

Table 3 *- Correlation Between Variables and Collagen Markers*

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Characteristic	C1M Correlation Coefficient	*P*	C4M2 Correlation Coefficient	*P*	C4Ma3 Correlation Coefficient	*P*	Pro-C5 Correlation Coefficient	*P*
Age, y	0.078	.117	-0.061	.220	0.054	.273	0.023	.642
Pack y	0.096	.056	0.073	.145	0.129	.010	0.100	.044
BMI, kg/m^2	-0.044	.372	-0.026	.594	0.036	.469	0.006	.899
BODE index	0.150	.003	0.152	.003	0.135	.008	0.159	.002
6MWT, m	-0.095	.062	-0.035	.489	-0.100	.048	-0.114	.023
Exacerbation rate	-0.10	.836	-0.023	.645	-0.035	.475	-0.056	.247
Severe exacerbation rate	-0.009	.190	0.049	.323	0.060	.221	0.049	.314
Unadjusted Charlson Score	0.106	.032	-0.060	.225	0.087	.076	0.076	.118
MMRC score	0.120	.016	0.113	.023	0.118	.017	0.108	.028
Lung function (post-brd)								
FEV_1, L	-0.115	.024	-0.075	.138	-0.081	.110	-0.104	.038
FVC, L	-0.030	.555	0.069	.174	0.009	.866	-0.011	.831
FEV_1, % pred	-0.152	.003	-0.100	.052	-0.146	.005	-0.139	.006
FVC, % pred	-0.038	.468	0.022	.675	-0.096	.063	-0.056	.271
FEV_1/FVC	-0.154	.002	-0.144	.004	-0.113	.026	-0.123	.014
Inflammation markers								
Copeptin, pmol/L	0.141	.112	0.070	.156	0.104	.035	0.141	.004
ADM, nmol/L	0.196	.0001	0.103	.037	0.166	.001	0.196	.0001
ANP, pmol/L	0.135	.182	0.016	.743	0.124	.012	0.135	.006
PCT, g/L	0.054	.436	0.051	.307	0.062	.212	0.054	.271

Boldface type highlights the statistically significant correlations. ADM = adrenomedullin; ANP = atrial natriuretic peptide; PCT = procalcitonin; pred = predicted. See Table 1 and 2 legends for expansion of other abbreviations.

Table 4 - Comparing the Biomarker Levels (Mean ± SD) in Patients Receiving and Not Receiving ICS

Biomarker	ICS (n = 346)	No ICS (n = 77)	*P*
C1M	34.5 ± 28.0	24.6 ± 11.6	**.002**
C4M2	58.8 ± 22.3	53.6 ± 17.2	.110
C4Ma3	6.3 ± 3.1	5.1 ± 2.2	**.002**
Pro-C5	530.7 ± 264.6	435.8 ± 172.4	**.004**

Boldface type highlights the statistically significant difference in collagen biomarkers between patients who had received ICS and those patients who had not. ICS = inhaled corticosteroids. See Table 2 legend for expansion of other abbreviations.

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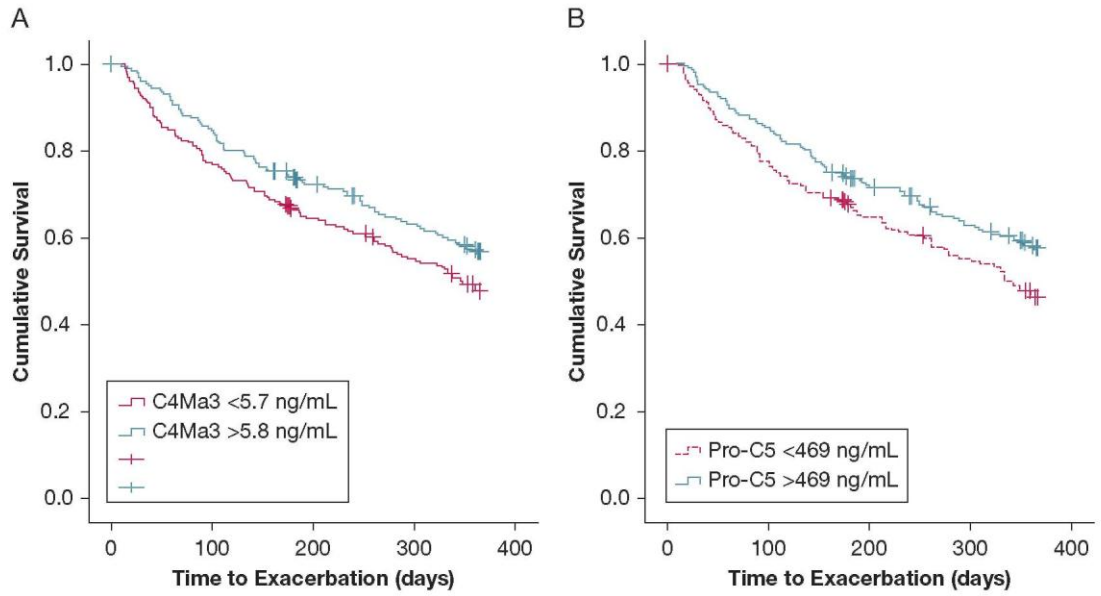
Figure 4 *- Kaplan-Meier curves showing the significant effect of (A) C4Ma3 (P = .05) and (B) Pro-C5 (P = .02) on time to exacerbation. See Figure 2 legend for expansion of abbreviations.*



Conclusion

Collagen degradation and formation may play a significant role in COPD severity (airflow limitation, dyspnea) and disease outcome (time to exacerbation, and prognosis as assessed by the BODE index).

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