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Characterization of serological neo-epitope biomarkers reflecting collagen remodeling in clinically stable chronic obstructive pulmonary disease

Jannie M.B. Sand [a,b,*], Gerd Martinez [c], Anne-Kirsten Midjord [c], Morten A. Karsdal [a], Diana J. Leeming [a], Peter Lange [b,c]

[a] Nordic Bioscience, Biomarkers and Research, Herlev, Denmark
[b] Section of Social Medicine, Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[c] Section of Respiratory Medicine, Hvidovre Hospital, Hvidovre, Denmark

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ABSTRACT

Objectives: Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation that leads to excessive remodeling of the lung extracellular matrix (ECM), resulting in release of protein fragments (neo-epitopes) to the blood. Serological markers assessing this have previously been associated with exacerbations of COPD. However, characterization of these in individuals with clinically stable COPD is lacking. The aim of this study was to characterize the collagen remodeling in stable COPD by the serological assessment of neo-epitopes.

Design and methods: Sixty-eight subjects with clinically stable COPD were included into the study at baseline, and 27 came back for a four weeks follow-up visit. Serum and plasma levels of neo-epitopes were assessed for the evaluation of collagen type III (C3M), IV (C4M, C4Ma3, P4NP 7S), and VI (C6M, Pro-C6) remodeling.

Results: C3M, C4M, C4Ma3, P4NP 7S, and C6M levels were significantly elevated in COPD subjects compared with healthy controls ($p < 0.0001$ to $p = 0.044$). Each neo-epitope biomarker was significantly correlated between serum and plasma ($p < 0.0001$) and most biomarkers were stable in the majority of patients from baseline to week four. Serum C6M levels were weakly correlated with FEV_1% predicted ($r = -0.274$, $p = 0.025$) and serum Pro-C6 levels were elevated in subjects with previous exacerbations ($p = 0.014$). C3M, C4Ma3, C6M, and P4NP 7S were weakly correlated with MRC dyspnea scores ($p < 0.01$). No associations were seen with BMI, smoking, duration of COPD, blood oxygen saturation, shuttle walk test distance, GOLD grades, or CAT scores.

Conclusions: Serological biomarkers of collagen remodeling were elevated in subjects with COPD as compared with healthy individuals. Biomarker levels were significantly correlated with measures of dyspnea, indicating a relationship with degree of symptoms, while only C6M showed a weak but significant association with lung function. Biomarker levels were not related to GOLD grades, which was in line with previous studies indicating that ECM remodeling may be related to disease activity rather than severity.

1. Introduction

The small airways undergo extensive remodeling in individuals with chronic obstructive pulmonary disease (COPD). Airway walls are thickened by inflammatory cell infiltrates, increased mucus glands, and fibrosis attributed to the accumulation of extracellular matrix (ECM). Together with the loss of elastic recoil and mucus plugging, the thickening of the airway wall is an important factor resulting in narrowing of the airway lumen [1] leading to the airway obstruction that characterizes COPD. Additionally, the destruction of alveolar tissue and small airways leads to emphysema [2]. The opposing mechanisms behind ECM accumulation and degradation and how these are interrelated, resulting in pathological changes characterizing COPD, are both intriguing and important to study. Hogg et al. have proposed that the small airway wall fibrosis precedes the emphysematous destruction of the alveolar tissue [3]. ECM composition in the lung has been the focus of only few studies although this seems to be the site of main pathological changes. Elastin expression is decreased in both alveolar tissue and small airways [2,4,5], whereas reports on collagen composition are more varied. Eurlings et al. found an elevated expression of fibrillar

* Corresponding author at: Nordic Bioscience, Biomarkers and Research, Herlev, Denmark.
E-mail address: jsa@nordicbioscience.com (J.M.B. Sand).

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collagens in alveolar and small airway walls in individuals with COPD compared to smoking controls [5]. On the other hand, Hogg et al. reported a decrease in the total bronchiolar tissue and a reduction in total collagen, but a relative increase in collagen type III over I [6]. Annoni et al. found collagen type I decreased in lungs of individuals with COPD as compared to non-smokers, but observed no difference in collagen type III and IV content [4].

In the present study, we wanted to investigate the remodeling of lung ECM in individuals with stable COPD, by serologically assessing neo-epitopes related to collagen synthesis and degradation in the systemic circulation.

2. Methods

2.1. Study design

Inclusion criteria were a diagnosis of COPD made by a senior physician and $FEV_1 < 80\%$ of predicted value. Exclusion criterion was an acute exacerbation of COPD leading to hospitalization within the previous four weeks. Sixty-eight subjects were included in the study from April 10th, 2014 to March 26th, 2015. Clinical information was collected by questionnaires and additional information was extracted from medical records. Collected data included basic demographics, spirometry, COPD history, smoking history, exacerbation history, COPD Assessment Test (CAT), Medical Research Council (MRC) dyspnea score, therapies for COPD, shuttle walk test, blood oxygen saturation (SAT), and comorbidities. Twenty-seven subjects also attended a second visit four weeks later. Blood sampling was performed at both visits. The study complies with the Declaration of Helsinki and Good Clinical Practice Guidelines, and has been approved by the local ethics committee (protocol number H-6-2013-014). All participants provided written informed consent before the performance of all study-related assessments.

Serum and plasma samples collected from healthy donors ($n = 16$) with no symptomatic or chronic disease were obtained from the commercial vendor Valley Biomedical (Wichester, Virginia).

2.2. Measurements of serological biomarkers

Whole blood was collected by venipuncture into vacutainer tubes (BD Vacutainer Clot Activator Tube, cat. no. 367896 for collection of serum, and BD Vacutainer Lithium Heparin PST II, cat. no. 367378 for collection of plasma; BD Diagnostics, Plymouth, UK). Serum was prepared by allowing the blood to clot for 1 h at room temperature followed by centrifugation at 4 °C at 2730 $min^{-1} \times g$ for 15 min. Plasma (heparin anticoagulant) was obtained by centrifugation under same conditions as serum, but without prior clotting. Serum and plasma were stored at −80 °C until analyzed. Degradation fragments of collagen type III (C3M) [7], IV (alpha-1 and alpha-3 chain by C4M and C4Ma3, respectively) [8], and VI (C6M) [9], and fragments released in relation to formation of collagen type IV (P4NP 7S) [10] and VI (Pro-C6) [11] were assessed serologically using validated immunoassays (Nordic Bioscience). The assays were run according to the manufacturer. Biomarker specifications are shown in Table 1.

2.3. Statistical analyses

COPD subject demographics were summarized as mean with standard deviation (SD), median with interquartile range (IQR), or number of subjects with percentage, as applicable. Biomarker levels were compared with clinical characteristics using the non-parametric Mann-Whitney or Kruskal-Wallis test. The paired Wilcoxon test was used to compare visit 1 with visit 2. Spearman's correlation coefficient was used to investigate the association of serum and plasma levels and the association with clinical parameters.

Table 1
Biomarker specifications.

Biomarker	Specifications	Measure	References
C3M	COL 3 degraded by MMPs	IM degradation	[7]
C4M	COL 4 (alpha-1) degraded by MMPs	BM degradation	[8]
C4Ma3	COL 4 (alpha-3) degraded by MMPs	Tissue specific BM degradation	[8]
C6M	COL 6 degraded by MMPs	IM degradation	[9]
P4NP 7S	COL 4 7S domain (internal epitope)	BM formation	[10]
Pro-C6	COL 6 C5 domain (neo-epitope)	IM formation	[11]

BM, basement membrane, COL, collagen; MMP, matrix metalloproteinase, IM, interstitial matrix.

3. Results

3.1. Basic demographics and clinical information

Sixty-eight subjects diagnosed with COPD and with Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades 2 to 4 were included in the study (Table 2). Mean age was 71 (range 43 to 90) years of age and 28 subjects (41%) were male. Enrolled subjects ranged from newly diagnosed (<one year) and subjects with a diagnosis made 48 years

Table 2
Basic demographics for clinically stable COPD subjects at baseline.

Parameters	n	Stable COPD
Demographics		
Age (yrs), mean SD	68	71 ± 9
Male/Female gender, %	28/40	41%/59%
BMI (kg/m^2), mean SD	61	24.5 ± 6.1
Duration of COPD (yrs), mean SD	68	10 ± 8
Pack years (yrs), mean SD	35	45 ± 29
Current/Ex/Never smokers, %	11/54/3	16%/79%/4%
Clinical variables		
GOLD 2/3/4, %	23/23/22	34%/34%/32%
GOLD A/B/C/D, %	5/13/6/44	7%/19%/9%/65%
FEV_1/FVC (%), mean SD	60	50 ± 14
FEV_1 (L), mean SD	62	0.989 ± 0.453
FEV_1 (% pred), mean SD	68	40.4 ± 16.3
FVC (L), mean SD	58	2.029 ± 0.676
FVC (% pred), mean SD	60	66.5 ± 17.4
SAT at rest (%), mean SD	63	95.1 ± 2.4
SAT following exercise (%), mean SD	40	89.8 ± 4.9
Incremental shuttle walk test (meters), mean SD	36	196 ± 85
MRC, median (IQR)	68	4 (3–5)
CAT, median (IQR)	68	17 (12−23)
Number of hospitalized exacerbations last year (0, 1–2, >2), %	42/19/7	62%/28%/10%
Interventions		
Oxygen supplement, %	10	15%
LABA, %	54	79%
LAMA, %	51	75%
SABA, %	36	53%
ICS, %	42	62%
Comorbidities		
No comorbidities	14	21%
Asthma, %	3	4%
Restrictive component, %	4	6%
Arthritis[a], %	14	21%
Osteoporosis, %	20	29%
Cardiovascular disease, %	21	31%
Hypertension, %	30	44%
Diabetes type 2, %	5	7%
Liver cirrhosis, %	1	1%

BMI, body mass index; CAT, COPD assessment test; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICS, inhaled corticosteroids; IQR, inter-quartile range; LABA, long-acting β_2-agonist; LAMA, long-acting muscarinic antagonist; MRC, Medical Research Council dyspnea scale; SABA, short-acting β_2-agonist; SAT, blood oxygen saturation; SD, standard deviation.

[a] Twelve subjects had osteoarthritis and two had inactive rheumatoid arthritis.