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Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease

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Summary
Background: Recent studies show that COPD patients exhibit low-grade systemic inflammation, and that plasma fibrinogen and high neutrophil counts are related to faster declines in lung function. We examined correlations between serum biomarkers and the decline of lung function in COPD patients.
Method: Baseline levels of 9 serum biomarkers (TIMP-1, α1-antitrypsin, MMP-9, TNF-α, TGF-β, IL-6, IL-8, neutrophil elastase and CRP), fibrinogen and white blood cell counts (WCC) were measured in 96 COPD patients. Lung function was measured at the time of blood sampling and every 3–6 months during the observation period (median 25.0 months).
Results: Twenty patients were rapid decliners of lung function and 53 patients were non-decliners. Neutrophil counts, serum CRP and MMP-9 were significantly higher in the rapid decliners ($p < 0.05$). The annual change of % predicted FEV_1 was inversely correlated with MMP-9 ($r = -0.288$; $p < 0.01$) and CRP ($r = -0.354$; $p < 0.005$) (partial correlation coefficients adjusted for age, sex, cardiovascular disease, smoking history, and baseline % predicted FEV_1). The remaining biomarkers were not correlated with the annual change of % predicted FEV_1.
Conclusion: Serum CRP and MMP-9 levels were related to FEV_1 decline. These markers are good candidates as predictors for rapid decline of FEV_1 in COPD patients. Additional long-term and larger size studies of COPD patients could help determine the exact roles for these biomarkers.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a slowly progressive disease induced primarily by smoking tobacco. FEV_1 is easily measured and is a spirometric predictor of mortality in patients with COPD.[1] Factors that affect the decline of FEV_1 are, therefore, of prognostic importance in COPD. Long-term studies of the natural history of COPD have shown the importance of tobacco smoking and exacerbation frequency.[2,3] Some smokers are more predisposed to rapid declines in lung function (''rapid decliners'') than others (''slow decliners'').[3–5] However, there is little information available regarding what factors contribute to the differences for these two types of patients.

Recent studies have shown that patients with COPD exhibit low-grade systemic inflammation[6,7] that is often associated with significant extra-pulmonary effects, such as cardiovascular abnormalities and skeletal muscle dysfunction.[6] Gan et al. performed a systematic review of these studies and found that circulating leukocytes, fibrinogen, serum c-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) levels were higher in COPD patients than in control subjects.[8] We previously reported that serum concentrations of tissue inhibitor of metalloproteinase-1 (TIMP-1), α1-antitrypsin (α1-AT), TNF-α, and IL-6 levels were elevated in COPD patients.[9,10] Circulating CRP levels have been reported as a predictor of mortality in COPD.[11,12] However, there is little information on how these inflammatory markers are related to lung function decline in COPD. Donaldson et al. reported that patients with high plasma fibrinogen levels and neutrophil counts had a faster % predicted FEV_1 decline.[13]

The aim of this study was to examine which inflammatory markers were related to a faster decline of FEV_1 in COPD patients, and also to examine the effect of smoking status and steroid treatment.

Methods

Subjects

COPD was defined according to the GOLD criteria.[14] COPD was diagnosed when the post-bronchodilator FEV_1/FVC (forced vital capacity) ratio was less than 70% (GOLD stages I–IV). A total of 96 patients with COPD were recruited from patients seen in the Department of Internal Medicine of the Wakayama Medical University Kihoku Hospital. Characteristics of the 96 COPD patients are shown in Table 1. All subjects were in a clinically stable condition, were not suffering from respiratory tract infections and did not experience disease exacerbation at least 4 weeks prior to blood sampling. Eleven patients were diabetic, 10 patients had cardiovascular disease, 4 patients had hyperlipidemia, 5 patients had chronic liver disease, 3 patients had gall bladder stones, and 8 patients had chronic gastritis. However, patients were excluded if they had bronchiectasis, tuberculosis or other confounding inflammatory diseases, such as malignancy, arthritis, connective tissue disorders or inflammatory bowel disease. The study was approved by the Ethics Committee of the Wakayama Medical University, and all patients gave informed consent for participation.

Table 1 Characteristics of 96 patients with COPD.

Characteristics[a]	Median	*N*	IQR
Observation period (months)	25.0		13.0–37.8
Age (years)	79		82.0–72.3
Males		95	
Smoking status			
Current smoker		24	
Non-current smoker		72	
Never smoked		0	
Smoking history (pack-years)	50.0		37.0–75.0
BMI	21.2		19.0–23.0
FEV_1/FVC ratio	58.4		50.8–65.1
FEV_1 (L)	1.5		1.0–2.0
FEV_1/% predicted	69.6		49.6–87.7
FVC (L)	2.5		2.0–3.1
COPD			
stage I		37	
stage II		35	
stage III		21	
stage IV		3	
Long-term oxygen therapy		20	

IQR: interquartile range.
[a] Measured at recruitment.

Measurements of hs-CRP, α1-antitrypsin, IL-8, IL-6, TNF-α, neutrophil elastase, TGF-β1, MMP-9 and TIMP-1

Serum high-sensitivity (hs) CRP was measured by immune nephelometry using the TBA-200FR NEO system (Toshiba Medical Systems; Tokyo, Japan), with a lower detection limit of $<$0.030 mg/dl. Serum α1-antitrypsin (α1-AT) was also determined using immune nephelometry.[10,15] Tumor necrosis factor (TNF)-α, interleukin (IL)-8, IL-6, TGF-β1, neutrophil elastase, Matrix metalloproteinase (MMP)-9 and TIMP-1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) kits (Amersham Biosciences AB, Uppsala, Sweden; BioLegend, San Diego, CA; Alexis, Lausen, Switzerland). All assays were performed according to the manufacturers' recommendations.

Categorization of smoking status

Subjects were categorized as current smokers, non-current smokers (former smokers) and those who had never smoked. To confirm smoking status, we measured serum cotinine levels using an enzyme-immunoassay (EIA) serum cotinine kit (Cozart Bioscience, Oxfordshire, UK). Cotinine is the main metabolite of nicotine. As significant serum concentrations of cotinine reflect exposure to tobacco smoke during the previous few days, measurement of serum cotinine allowed the objective classification of non-current and current smokers. A cotinine concentration $>$25 ng mL^{-1} was considered positive for smoking.[10,16]

Pulmonary function tests

Pulmonary function tests (Chestac-8800, Chest Ltd, Tokyo, Japan) were performed within 1 month prior to blood

sampling and every 3–6 months for more than 6 months during the observation period (median 25.0 months; interquartile range [IQR] 13.0–37.8 months). Lung function was assessed as % predicted FEV_1, with FEV_1 adjusted for age, height and sex. Rapid decliners had a decrease in % predicted $FEV_1 \geq 3.0\%$/year and non-decliners had an increase in % predicted $FEV_1 \geq 0.4\%$/year.[4,5]

Visual emphysema score

Pulmonary emphysema was assessed visually by 3 expert chest radiologists who were blinded to the clinical and lung function data, as described by Goddard et al.[17] Based on 3 high-resolution computed tomography (HRCT) slices (at the level of the carina, and at 5 cm above and below the carina), lung parenchyma were assessed for the extent of emphysema. The 3 HRCT slices were graded and scored separately for the left and right lungs, giving a total of 6 lung fields. The extent of emphysema using this direct observation method was scored on a 4-point scale. The extent score was 1 if <25% of the lung field was involved, 2 if there was 25–50% involvement, 3 for 50–75% involvement, and 4 for >75% involvement. The sum of the extent scores for the 6 lung fields had a possible maximum value of 24. The average score given by the 3 radiologists was used.

Statistical analysis

Patients' characteristics recorded at recruitment are given as medians (interquartile range [IQR]) (Table 1). Results are shown as the mean ± standard error of the mean (SEM). Comparisons between groups were done using unpaired Student's *t*-tests. Comparisons between COPD stages were made using analysis of variance (ANOVA), followed by Fisher's protected least-significant difference (PLSD) test. Correlation analysis used partial correlation coefficients adjusted for age, sex, cardiovascular disease, smoking history (pack-years) and baseline FEV_1 % predicted. Stepwise multiple regression analysis was done for any independent variable that showed significant predictor value in the univariate analysis; a *p*-value >0.25 was used for variable removal. A *p*-value <0.05 was considered statistically significant.

Results

Patient characteristics

The 96 patients (95 men) studied had mild to very severe COPD (Table 1). Of these, 20 patients were receiving home oxygen therapy, 10 were receiving inhaled steroids daily, 5 were receiving oral steroids and one patient received both kinds of steroids. Four patients died during the follow-up period. Three patients were receiving non-invasive positive pressure ventilation (NIPPV).

Annual changes of lung function

On average, the annual changes of FEV_1 and the FEV_1/FVC ratio declined by 20.2 mL/year and 0.41%/year, respectively. The average annual changes for % predicted FEV_1 and FVC increased by 0.94%/year and 8.8 mL/year. Twenty patients were rapid decliners of lung function, whose decline of % predicted FEV_1 was >3%/year ($\Delta FEV_1 = -179 \pm 29.4$ mL/year), and 53 were non-decliners ($\Delta FEV_1 = +58.8 \pm 11.4$ mL/year).

Correlations between annual changes of lung function and biomarkers

The annual changes of % predicted FEV_1 were significantly correlated with serum MMP-9 ($r = -0.288$; $p < 0.01$) and CRP ($r = -0.354$; $p < 0.005$) (partial correlation coefficients adjusted for age, sex, cardiovascular disease, smoking history and baseline % predicted FEV_1) (Table 2). These markers were also inversely correlated with the annual changes of FEV_1 values (MMP-9: $r = -0.277$, $p < 0.05$; CRP: $r = -0.303$, $p < 0.01$). These biomarkers were not correlated with the annual changes of other lung function parameters, including FVC, VC and % predicted VC. The remaining biomarkers were not significantly correlated with the annual changes for FEV_1, % predicted FEV_1 or FVC.

Stepwise multiple regression analysis was done for any independent variable that showed significant predictor value from the univariate analyses (MMP-9 and CRP). MMP-9 and CRP were independent predictors of the annual changes of % predicted FEV_1 (R^2 for the overall model was 0.14, $p < 0.005$). Thus, only a small proportion of the variance (14%) for the annual change of % predicted FEV_1 was explained by CRP and MMP-9 concentrations.

Neutrophil counts and serum MMP-9 and CRP levels were significantly higher in rapid decliners of FEV_1 compared to non-decliners (Table 3). The remaining biomarkers and BMI were not significantly different between the 2 groups.

Table 2 Adjusted correlation coefficients for annual changes of % predicted FEV_1 with biomarkers and emphysema scores in COPD patients.

Parameters	*N*	Adjusted correlation coefficient (*r*)	*p*-Value
BMI (kg/m^2)	96	0.099	0.357
WCC ($109\ L^{-1}$)	76	−0.207	0.087
Neutrophil counts ($109\ L^{-1}$)	28	−0.394	0.057
Fibrinogen ($mg\ L^{-1}$)	52	−0.286	0.054
MMP-9 ($ng\ mL^{-1}$)	90	−0.288	0.008[a]
TNF-α ($pg\ mL^{-1}$)	75	0.142	0.245
IL-6 ($pg\ mL^{-1}$)	84	0.015	0.897
TGF-β ($ng\ mL^{-1}$)	89	−0.214	0.054
TIMP-1($ng\ mL^{-1}$)	88	0.005	0.961
Elastase ($ng\ mL^{-1}$)	56	−0.160	0.266
α1-AT ($mg\ L^{-1}$)	87	−0.138	0.221
IL-8 ($pg\ mL^{-1}$)	90	−0.022	0.845
hs-CRP ($mg\ L^{-1}$)	84	−0.354	0.002[a]
Goddard score	47	−0.143	0.373

Adjusted for age, sex, cardiovascular disease, smoking history (pack-years), and baseline FEV_1 % predicted.
WCC: white blood cell count, BMI: body mass index.
[a] Significant correlation with annual change of % predicted FEV_1.

Baseline lung function, visual emphysema scores (Goddard scores) and annual changes of FEV_1

Baseline lung function parameters (FEV_1, % predicted $FEV_1$and FVC) were not correlated with the annual changes of % predicted FEV_1 ($r = -0.060$, $p = 0.574$; $r = -0.104$, $p = 0.328$; $r = -0.047$, $p = 0.663$, respectively) (partial correlation coefficients adjusted for age, sex, cardiovascular disease, smoking history), and were not significantly different between rapid decliners and non-rapid decliners (data not shown). The annual FEV_1 decline tended to be greater in mild disease than severe disease, although the differences were not significant (GOLD stage I: -26.1 ± 27.9 mL/year; stage II: -25.2 ± 21.9 mL/year; stage III: -10.8 ± 17.3 mL/year; stage IV: 47.5 ± 21.4 mL/year). The annual changes of % predicted FEV_1 were not different by GOLD stages.

Emphysema was evaluated by HRCT in 47 patients. Goddard scores at the start of the study period were not correlated with the annual changes of % predicted FEV_1 (Table 2). Goddard scores were not different between rapid decliners and non-decliners (Table 3). Serum α1-AT levels were significantly correlated with Goddard scores ($r = 0.3926$, $p = 0.0084$).

Influence of smoking status on biomarkers and lung function changes

Serum levels of TNF-α ($p = 0.010$) and IL-8 ($p = 0.025$) were significantly higher in current smokers compared to non-current smokers (Table 4). The other biomarkers were not affected by smoking status. Annual changes of lung function parameters (FEV_1, % predicted FEV_1 or FVC) were not different between current smokers and non-current smokers. There was no correlation between smoking history (pack-years) and the annual changes of FEV_1 or % predicted FEV_1 (Table 2), although the extent of FEV_1 decline for current smokers tended to be greater than that of non-current smokers (Table 4). Smoking history (pack-years) was not different between rapid decliners and non-decliners (71 ± 18 and 57 ± 5, $p = 0.195$).

Effect of inhaled or oral steroid treatment on biomarkers and annual changes of lung function

Inhaled or oral steroid treatment had no significant effect on lung function changes. Annual changes of FEV_1 or % predicted FEV_1 were not different between the patients with and without steroid treatments (Table 5). WCC and IL-8 serum levels were significantly higher in the patients receiving steroids compared to those without steroid treatment. The levels of other biomarkers were not significantly different between the COPD patients with and without steroid treatment.

Discussion

Serum CRP and MMP-9 levels and circulating neutrophil counts were related to an accelerated rate of FEV_1 decline in COPD patients. However, the available data are limited regarding blood biomarkers as predictors of lung function decline in COPD. Donaldson et al. reported that high plasma fibrinogen levels and circulating neutrophil counts were associated with a faster % FEV_1 decline in COPD.[13] This is consistent with our results that neutrophil counts were higher in rapid decliners compared to non-decliners. They also showed that frequent exacerbators have faster rises in airway and systemic inflammatory markers. Frequent exacerbators, with an exacerbation frequency of >2.52 exacerbations per year, were more likely to show a faster rise in plasma fibrinogen and sputum IL-6 over time than patients with a history of infrequent exacerbations.[13]

These results suggest that increased airway inflammation, due possibly to frequent exacerbations, is associated with a faster lung function decline in patients with COPD. However, the primary cause for systemic inflammation in

Table 3 Biomarkers and emphysema scores: Comparisons between COPD rapid decliners and non-decliners of lung function.

Parameters	Non-decliners		Rapid decliners		*p*-Value
	Mean ± SE	*n*	Mean ± SE	*n*	
Age (years)	77.6 ± 1.1	53	78.6 ± 2.0	20	0.655
BMI (kg/m^2)	21.2 ± 0.4	53	21.5 ± 0.9	20	0.751
WCC (10^9 L^{-1})	6.44 ± 0.30	40	7.31 ± 0.59	18	0.156
Neutrophil counts (10^9 L^{-1})	3.31 ± 0.24	15	4.80 ± 0.82	6	0.030[a]
Fibrinogen (mg L^{-1})	304 ± 12	27	345 ± 19	14	0.069
MMP-9 (ng mL^{-1})	197 ± 19	48	285 ± 49	20	0.046[a]
TNF-α (pg mL^{-1})	7.44 ± 0.34	41	7.32 ± 0.56	16	0.861
IL-6 (pg mL^{-1})	4.66 ± 1.06	46	5.11 ± 1.42	18	0.812
TGF-β (ng mL^{-1})	50.5 ± 2.5	47	57.6 ± 3.4	20	0.109
TIMP-1(ng mL^{-1})	201 ± 6	46	208 ± 9	20	0.520
Elastase (ng mL^{-1})	106 ± 13	27	132 ± 26	14	0.315
α1-AT (mg L^{-1})	146 ± 5	46	144 ± 5	18	0.858
IL-8 (pg mL^{-1})	13.3 ± 1.5	48	13.5 ± 1.7	20	0.925
hs-CRP (mg L^{-1})	0.21 ± 0.04	43	0.56 ± 0.23	20	0.042[a]
Goddard score	8.62 ± 1.27	21	8.54 ± 1.95	13	0.971

Non-decliner: increase in % predicted $FEV_1 \geq 0.4$%/year, Rapid decliner: decrease in % predicted $FEV_1 \geq 3$%/year.
[a] Significant difference by unpaired *t*-test.

Table 4 Influence of smoking status on annual changes of lung function, biomarkers and emphysema scores.

Parameters	Non-current smokers		Current smokers		*p*-Value
	Mean ± SE	*n*	Mean ± SE	*n*	
FEV_1 annual change (mL/year)	−15.5 ± 16.6	58	−41.1 ± 27.9	32	0.402
% predicted					
FEV_1 annual change (%/year)	1.00 ± 0.96	57	0.20 ± 1.34	31	0.626
BMI (kg/m^2)	21.2 ± 0.4	58	20.4 ± 0.8	31	0.321
WCC ($10^9\ L^{-1}$)	6.49 ± 0.27	51	6.73 ± 0.50	25	0.643
Neutrophil counts ($10^9\ L^{-1}$)	3.60 ± 0.33	20	3.89 ± 0.70	8	0.674
Fibrinogen ($mg\ L^{-1}$)	321 ± 13	32	361 ± 30	16	0.149
MMP-9 ($ng\ mL^{-1}$)	219 ± 21	58	202 ± 32	32	0.635
TNF-α ($pg\ mL^{-1}$)	6.91 ± 0.32	53	8.35 ± 0.31	22	0.010[a]
IL-6 ($pg\ mL^{-1}$)	4.10 ± 0.54	56	5.97 ± 1.72	28	0.193
TGF-β ($ng\ mL^{-1}$)	53.9 ± 1.9	58	51.8 ± 3.6	31	0.551
TIMP-1 ($ng\ mL^{-1}$)	193 ± 6	58	213 ± 8	30	0.056
Elastase ($ng\ mL^{-1}$)	117 ± 12	42	117 ± 23	14	0.980
α1-AT ($mg\ L^{-1}$)	142 ± 4	55	143 ± 4	27	0.778
IL-8 ($pg\ mL^{-1}$)	11.5 ± 0.7	58	15.7 ± 2.1	32	0.025[a]
hs-CRP ($mg\ L^{-1}$)	0.24 ± 0.07	54	0.36 ± 0.10	30	0.370
Goddard score	9.27 ± 1.03	37	8.20 ± 2.36	10	0.648

[a] significant difference by unpaired *t*-test.

patients with COPD remains to be elucidated. Donaldson et al. examined the relationship between airway inflammation and systemic inflammation. There was no relationship between sputum IL-6 and plasma fibrinogen, suggesting that there is no direct link between systemic and airway inflammation. Another study also showed that these two compartments were not related. Vernooy reported that there were no direct correlations between sputum and plasma sTNF receptors in patients with COPD.[18]

These results suggest that systemic inflammation in COPD is not due to an overflow of inflammatory mediators from the local compartment. Rather, this implies that the inflammatory responses in the local and systemic compartments are regulated differently. Systemic hypoxia has been suggested to be a good candidate, as systemic hypoxia is associated with activation of the TNF-α system in patients with COPD. In line with this hypothesis, Takabatake and colleagues recently reported that systemic hypoxia observed in patients with COPD, due to deterioration of lung function, might contribute to enhanced levels of systemic inflammatory markers.[19] However, systemic hypoxia will usually occur only in very severe disease. Additional studies are clearly required to elucidate the origin and regulation of the systemic inflammatory response in COPD.

MMP-9 has been implicated in human emphysema, with its principal effect being the destruction of the extracellular matrix, particularly elastin.[20] The protease:antiprotease hypothesis has dominated thinking regarding the pathogenesis of emphysema. Studies of human samples

Table 5 Influence of inhaled or oral steroids on annual changes of FEV_1, % predicted FEV_1 and biomarkers.

Parameters	Without steroids		With steroids		*p*-Value
	Mean ± SE	*n*	Mean ± SE	*n*	
FEV_1 annual change (mL/year)	−25.2 ± 15.7	82	9.0 ± 23.9	14	0.389
% predicted					
FEV_1 annual change (%/year)	0.84 ± 0.85	82	1.55 ± 1.15	14	0.735
WCC ($10^9\ L^{-1}$)	6.23 ± 0.23	64	8.39 ± 0.73	12	0.001[a]
Fibrinogen ($mg\ L^{-1}$)	322 ± 14	44	366 ± 23	8	0.212
MMP-9 ($ng\ mL^{-1}$)	203 ± 18	76	269 ± 51	14	0.171
TNF-α ($pg\ mL^{-1}$)	7.17 ± 0.29	62	8.15 ± 0.57	13	0.149
IL-6 ($pg\ mL^{-1}$)	4.58 ± 0.76	71	5.50 ± 1.44	13	0.626
TGF-β ($ng\ mL^{-1}$)	51.9 ± 1.9	75	60.2 ± 3.5	14	0.081
TIMP-1 ($ng\ mL^{-1}$)	199 ± 6	74	207 ± 9	14	0.542
Elastase ($ng\ mL^{-1}$)	114 ± 11	48	136 ± 30	8	0.462
α1-AT ($mg\ L^{-1}$)	143 ± 3	74	145 ± 7	13	0.738
IL-8 ($pg\ mL^{-1}$)	12.3 ± 0.6	76	17.1 ± 4.6	14	0.047[a]
hs-CRP ($mg\ L^{-1}$)	0.245 ± 0.06	71	0.475 ± 0.244	13	0.184

[a] Significant difference by unpaired *t*-test.

show increases in many proteases, including MMP-1, -2, -8, -9, and -14 (MT1-MMP) in smoking-related emphysema.[21] Lung parenchyma destruction by elevated MMP-9 may contribute to additional airflow limitations (FEV_1 decline) by decreasing lung elastic recoil. In the normal lung, MMP-9 is not produced by resident cells. However, under various forms of stimulation, numerous cell types in the lung will produce MMP-9. These include bronchial epithelial cells, Clara cells, alveolar type II cells, fibroblasts, smooth muscle cells, endothelial cells, leukocyte, lymphocytes, eosinophils, mast cells, NK cells, dendritic cells and macrophages.

Thus, many types of lung cells may contribute to MMP-9 production under pathological conditions. Pinto-Plata et al. compared the serum proteomic profiles for 143 serum biomarkers in patients with COPD with those from age and sex-matched controls using a novel protein microarray platform (PMP) technology.[22] By univariate analyses, 43 biomarkers, including TIMP-1 and MMP-9, were identified that differed between patients and controls. Among these markers, serum MMP-9 showed the strongest correlation with exacerbation rates. They suggested that MMP-9 may be related to the rapid decline of FEV_1 with regard to the rate of exacerbation. Our results support this hypothesis. However, the source(s) of circulating MMP-9 for COPD patients remains to be elucidated.

CRP is the most well-studied biomarker of systemic inflammation in COPD.[12] CRP levels in stable COPD are elevated, and are correlated with arterial oxygen tension and 6-min walk distance.[11] However, the predictive value of CRP for COPD prognosis is still controversial. Gan and coworkers were the first to note the importance of high CRP levels in COPD patients. They showed that CRP is elevated in patients who are active smokers, had reduced lung function or had stable COPD. They also demonstrated that for patients with COPD, CRP levels predicted cardiovascular mortality, and decreased after treatment with inhaled corticosteroid.[8,23]

A role for CRP levels as a predictor of cardiovascular and global mortality in the general population has been postulated, but has not yet been fully accepted.[24] Two epidemiologic studies showed that increased CRP levels were independently associated with global and cardiovascular mortality in COPD patients with mild-to-moderate degrees of airway obstruction.[12,25] In the first study for individuals from the Lung Health Study with mild-to-moderate airway obstruction,[25] Man et al. found that patients in the highest CRP quartile had the highest risk of all-cause mortality. Recently, Dahl et al., using data from the Copenhagen City Heart Epidemiologic Study, found that the CRP level was a strong, independent predictor of COPD outcomes represented by hospitalization and death.[12] However de Torres et al. reported that CRP levels were not associated with mortality in moderate-to-severe COPD patients.[26] They speculated that this discrepancy was due to the differences of severity for the subjects in these studies.

The 2 former studies used mild-to-moderate COPD with few or no symptoms in contrast to the latter report that studied moderate-to-severe disease. CRP is an acute-phase reactant that increases in a very sensitive, but non-specific way for most forms of tissue damage, inflammation and infection, all of which are very dynamic processes in patients with COPD. Further, CRP levels can be influenced by many different factors, including cardiovascular disease,[27] degree of physical activity, diabetes,[28] renal disease, hypertension, metabolic syndrome, obstructive sleep apnea, smoking status and treatments, such as statins. These confounding factors may influence the results when we discuss the value of CRP as a predictor for prognosis in COPD. In our study, 11 patients were diabetic and 10 patients had cardiovascular disease. These conditions may have influenced the results.

Emphysema scores and lung function parameters at the beginning of the study were not related to the annual changes of FEV_1. However, patients with mild-to-moderate disease tended to have faster declines compared to the patients with severe to very severe disease, although the differences were not significant. This is consistent with a previous study that found that patients with a high starting value for % predicted FEV_1 had a faster decline.[13] This is contrary to the conventionally accepted idea that a high initial FEV_1 is related to a slow decline, and that a low initial FEV_1 is related to a fast decline. Fletcher et al.[29] cautioned against this assumption in their landmark monograph. A slower decline in a group of patients with very severe COPD may be due, in part, to a survival effect, with faster decliners absent due to death or the use of intensive therapy in the latter stages of the disease.

Smoking status was not related to the annual changes of FEV_1 in this study, although the annual FEV_1 decline for current smokers tended to be greater than that of non-current smokers. It has been suggested that there is a dose—response relationship between the number of pack-years smoked and lung function decline.[30] Subjects who continued to smoke experienced steeper rates of decline compared with men who never smoked. After quitting, their rates of decline diminished to a level similar to that of men who had never smoked. FEV_1 decline in continuing smokers was significantly associated with duration of smoking, intensity and pack-years. The Lung Health Study (LHS) also showed that smoking cessation early in the course of COPD can slow the rate at which lung function is lost.[31] After 5 years, smoking cessation significantly reduced the rate of decline in lung function, although the use of a bronchodilator did not influence the long-term decline of FEV_1.

In our study, serum levels of TNF-α and IL-8 were higher in current smokers than in non-current smokers. Previous studies showed that active cigarette smoking, which is a leading risk factor for decreased FEV_1, can also independently induce systemic inflammation. Gan et al. examined the effects of active smoking and lung function decline on the elevation of systemic inflammatory markers (CRP, fibrinogen, and leukocyte) using 7685 adult participants in the Third National Health and Nutrition Examination Survey.[23] They found an additive effect of active smoking and reduced FEV_1 for these markers, suggesting their potential interactions in the pathogenesis of systemic complications observed in patients with poor lung function.

The annual changes of FEV_1 were not different by use of inhaled corticosteroid (ICS) or oral steroid in this study. The effectiveness of ICS in patients with COPD remains controversial. Randomized controlled trials, meta-analyses, medication withdrawal studies and observational reports have examined this question, with mixed results.

Two meta-analyses of the results from 4 large studies together with several other smaller trials reported contradictory conclusions.[32,33]

One group concluded that ICS reduced FEV_1 decline by a small, but statistically significant mean rate of 7.7 mL/year, whereas a second group concluded that the reduction in the mean rate was only 5.0 mL/year and was not statistically significant. Sin et al. reported that 2 weeks use of inhaled fluticasone or oral prednisone significantly reduced CRP levels from baseline levels in 41 mild-to-moderate COPD patients, and suggested the potential use of these drugs for improving cardiovascular outcomes in COPD.[34] However, they recently reported that neither fluticasone nor the combination of fluticasone/salmeterol (4 weeks) had a significant effect on CRP or IL-6 levels in a larger double-blind randomized placebo-controlled multicenter trials (289 patents).[35] They speculated that the discrepancy between the studies arose from the difference of subjects' backgrounds, including disease severity, smoking status and former ICS use.

In the same study, they showed that fluticasone reduced circulating SP-D levels compared to placebo, and that, conversely, withdrawal of the drug increased SP-D levels. They suggested that ICS reduced lung-specific, but not generalized biomarkers of systemic inflammation in COPD, such as CRP. In our study, CRP and IL-6 levels were not different by use of ICS. However, peripheral white blood cell counts and IL-8 serum levels were greater in COPD patients receiving steroid treatments than in those without steroids. Glucocorticoids are known to cause a marked increase in circulating polymorphonuclear (PMN) cells.[36] Thus, steroid treatment may increase PMN in COPD. Background disease status may influence circulating PMN rather than the steroid treatment, because we used steroids mostly for severe disease or for those patients with asthmatic components.

This study was limited by its observational nature, the short observation period and the relatively small number of subjects, especially for discussion of the absolute outcomes of COPD (mortality). We measured biomarkers at a single point only. As suggested by Donaldson, longitudinal studies are needed to confirm the reliability of these markers and their relationships to other clinical factors.[13] Our data set is incomplete. Inflammatory markers and visual emphysema scores (HRCT) were not available for all patients. This may have caused a type II error in the statistical analysis. Multiple backward stepwise regressions showed that MMP-9 and CRP were independent predictors of annual changes of FEV_1. However, only a small proportion of the total variance (14%) for annual changes of FEV_1 was explained by these markers. Additional large scale studies with longer observation periods will be required.

In summary, we have shown for the first time that high levels of circulating MMP-9 and CRP, as well as neutrophil counts, are associated with rapid decline of FEV_1 in COPD. These results suggest that these circulating biomarkers are good candidates as predictors for rapid decline of FEV_1 in COPD, although a larger size study with longer term observation is needed to confirm these results.

Conflict of interest

The authors declare that none of them has any conflict of interest related to the article or the research described.

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