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Blood biomarkers and measures of pulmonary function—A study from the Swedish twin registry

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Summary

Objective: There is great need of biomarkers for research and clinical purposes in COPD. This study explored the relationships between ten putative plasma biomarkers of COPD and physiological measures of reduced lung function.

Methods: FEV_1, FVC, residual volume/total lung capacity (RV/TLC) and CO diffusion capacity (D_LCO) were assessed in 357 subjects from the Swedish Twin Registry. The lung function measures were studied in relation to plasma levels of desmosines, C-reactive protein (CRP), plasminogen inhibitor activator (PAI-1) concentration and activity, tissue inhibitor of metalloproteinase (TIMP-1), clara cell protein 16 (CC16), surfactant protein D (SPD), matrix metalloproteinase 9 (MMP-9), hepatocyte growth factor (HGF) and interleukin (IL)-8.

Results: After adjustments for age, sex, height, BMI and smoking, FEV_1 was significantly associated with PAI-1 activity and desmosines. RV/TLC was significantly associated with CC16, PAI-1 concentration and PAI-1 activity, and D_LCO was significantly associated with desmosines, TIMP-1 and CRP. When the multivariate analysis was restricted to subjects with COPD (i.e., FEV_1/FVC < 0.70), CRP and desmosines were inversely associated with lung function.

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Conclusion: Several biomarkers were associated with lung function in this cross-sectional study. Especially CRP and desmosines could be useful markers to assess disease severity in subjects with COPD.

Introduction

Lung function in adults, as measured by spirometry, is characterized by slow and irreversible age-related decline. The rate of decline is accelerated in some individuals, e.g., due to smoking, environmental exposures or individual susceptibility. Chronic obstructive pulmonary disease (COPD) develops slowly and is usually the result of many years of progressive lung function decline. Today, there are few possibilities to monitor this process, besides repeated examinations of lung physiology. There is great need for biomarkers that could be used as sensitive measures of the disease activity and assist as predictors of lung function decline.[1]

Many blood biomarkers have been proposed in COPD, and the markers are assumed to measure different parts of the disease process. C-reactive protein (CRP), fibrinogen, interleukin (IL)-6, IL-8, i.e., unspecific markers of systemic inflammation, have been related to COPD and/or reduced FEV_1.[2–7] These markers could hypothetically reflect the inflammatory activity in the lungs, and thereby disease progression. Plasma levels of clara cell protein 16 (CC16) and surfactant protein D (SPD) have been considered as more lung specific markers, since these proteins are secreted from cells in the bronchial epithelium.[8,9] Desmosines, matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) are assumed to be associated with the degradation of lung tissue.[5,7] Desmosines are linked to the elastic fibers and released to the circulation as a result of elastin degradation. We recently reported that desmosines in plasma and urine were associated with several physiological measures of lung function.[10] Hepatocyte growth factor (HGF) has been suggested as a factor related to tissue repair in pulmonary diseases.[11,12] Plasminogen activator inhibitor 1 (PAI-1) is an inhibitor of fibrinolysis and serves as a pro-thrombotic factor. PAI-1 is associated with inflammation and features of the metabolic syndrome.[13] It was recently reported that COPD is associated with increased PAI-1 concentrations in plasma and sputum.[14]

The purpose of the present study was to explore ten plasma biomarkers, previously associated with COPD or reduced lung function, by studying the relationships between the biomarkers and different physiological measures of lung function in a population-based sample of Swedish twins.

Methods

Study population

The study was based on the Swedish Twin Registry (STR), which contains information on more than 80,000 twin pairs

($n = 160,000$) born from 1886 to 2000. Between 1998 and 2002, all living twins in the STR born in 1958 or earlier were contacted using a computer-assisted telephone interview developed for the SALT (Screening Across the Lifespan Twin) study.[15] The interview included a checklist of common diseases and respiratory symptoms, as well as smoking habits. From the population of 26,516 twin pairs ($n = 53,032$) where both participated in the telephone interview, 1030 twins in 515 pairs were selected to participate in more in-depth measurements of lung function. To assure that the sample would contain twins with symptoms of respiratory disease (self-reported symptoms of cough, chronic bronchitis, emphysema or asthma) disease concordant and discordant twins were prioritized over symptom free twin pairs. The selection of subjects and lung function testing procedures has been described previously.[16] In total, 392 twins (38%) of 1030 twins accepted the invitation to participate. Of them, acceptable measurements of forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), residual volume/total lung capacity (RV/TLC) and carbon monoxide diffusing capacity (D_LCO), were obtained for 357 individuals. Based on the answers from the telephone interview and subsequent questionnaire at the clinic, smoking status was defined as current smoker, ex-smoker, never-smoker or occasional (social) smoker. The number with information about plasma biomarkers ranged from 246 (for HGF) to 347 (for CRP). Of the 357 study subjects, there were 87 pairs of monozygotic twins, 39 dizygotic pairs of same-sex, 37 twin pairs of opposite sex and 31 unmatched twins. The study was approved by the Ethics Committee at Karolinska Institutet, No.03-461. All subjects signed informed consent.

Determination of blood biomarkers

Plasma was prepared from blood, collected in 10-mL Vacutainer tubes with EDTA, by centrifugation at 500 *g* for 10 min at room temperature. For the preparation of serum, blood was collected in 10-mL Vacutainer tubes and allowed to stand at room temperature for 1 h. It was then centrifuged at 500 *g* for 10 min at room temperature.

The desmosine data have been reported previously but are included here for comparison.[10] The concentration of the sum of total (free plus peptide bound) desmosine and isodesmosine in plasma (desmosines) was measured by liquid chromatography combined with tandem mass spectrometry.[10] The lower and upper limits of quantification were 0.1 and 2.0 nmol/L, respectively, with coefficients of variation of 5–6% at the low end and 8–9% at the high end.

C-reactive protein was measured with a high-sensitive assay (hsCRP) using near infrared particle immunoassay

(Turbidimetry NIPIA) with a detection range of 0.2—380 mg/L.

Human clara cell protein (CC16) (range 2—100 ng/mL) and human surfactant protein D (SPD) (range 1.56—100 ng/mL) were measured in plasma using ELISA immunoassays from BioVendor Laboratorni medicina, a.s., Modrice, Czech Republic.

Human hepatocyte growth factor (HGF) (range 10—10,000 pg/mL), interleukin-8 (IL-8) (range 2.4—2500 pg/mL), and tissue inhibitor of metalloproteinase 1 (TIMP-1) (range 55—40,000 pg/mL) were measured in plasma using an MSD® 96-well multi-array system from Meso Scale Diagnostics (Gaithersburg, Maryland, USA). Matrix metalloproteinase 9 (MMP-9) was measured in serum in the range 0.12—500 ng/mL using the same system from Meso Scale Diagnostics.

The plasma concentration of plasminogen activator inhibitor type 1 (PAI-1) was measured by a Quantikine ELISA immunoassay from R&D Systems (Minneapolis, MN, USA) in the range 0.31—20 ng/mL. The PAI-1 activity in plasma was measured by a functional assay ELISA kit (range 3.12—220 U/mL) from Molecular Innovations, Novi, Michigan, USA.

Some individuals had plasma biomarker concentrations below the lowest level of quantification (LLOQ). If the number below LLOQ was low, a value corresponding to 50% of the LLOQ was assigned. This procedure was used for CC16 in 24 cases, for TIMP-1 in 3 cases and for SPD in one case. For PAI-1 activity 137 cases (45%) were below LLOQ and these were grouped in a separate category (see statistics section).

Statistical methods

The median and range for levels of biomarkers were calculated and presented for descriptive purposes. Multiple linear regression was used to explore whether the following factors were associated with biomarker concentrations: age, gender, height, smoking status and BMI. Gender and smoking (current and occasional smokers vs. never smokers and former smokers) were modeled as dichotomous variables, age, height and BMI were modeled as continuous variables, and levels of the biomarkers were fitted as dependent variables.

Multiple linear regression was also used to assess the relationship between biomarkers and lung function measures, with lung function measures as the dependent variables. A separate analysis of the relationship between biomarkers and lung function was conducted for those with and without COPD. In both analyses, the data were adjusted for confounding factors, i.e. age, gender, height, smoking and BMI.

Due to positively skewed distributions, log-transformed (natural logarithm) values of desmosines, CRP, MMP-9, SPD, IL-8, PAI-1 concentration and HGF were used in all multiple regression analyses. CC16 and TIMP-1 were not log transformed, since the distributions were close to normal. The relationships between lung function measures and biomarkers are presented as standardized beta-coefficients.

The lung function values were also studied in relation to quartiles of the biomarker concentrations. The study population was divided into quartiles according to the concentrations of each biomarker, with equal numbers of men and women in each quartile. The mean lung function values were compared across the quartiles, after adjustment for confounding factors in a general linear model.

Since a high proportion of the subjects (137/304) had PAI-1 activity below the lowest level of quantification (LLOQ), we did not use linear regression to study the PAI-activity. Instead, PAI-1 activity was categorized. Subjects with PAI-1 activity below LLOQ formed a reference category and those with PAI-1 activity above LLOQ were divided into quartiles. The relationships between categories of PAI-1 activity and lung function measures were assessed for significance in a general linear model. *P*-values were computed for the overall relationship with PAI-1 activity and for the difference between the highest and lowest category.

The relationship between concentrations of biomarkers and lung function was also studied within twin pairs of the same gender. The biomarker and lung function values of the first twin were subtracted from the corresponding values in the second twin. Relationships between the differences were assessed using Spearman rank correlations.

Results

Study cohort

A total of 357 individuals (131 men, 226 women) had complete information on lung function and at least one of the blood biomarkers. Mean age was 59.1 ± 8.5 years. Age, gender, and COPD status of the participants in relation to smoking are presented in Table 1. The number of subjects with information about the various biomarkers ranged between 246 and 347 (Table 1).

Factors associated with concentrations of biomarkers

Multiple linear regressions were performed, with the biomarker as dependent variable, and age, sex, height, BMI and smoking as independent variables (Table 2). Except for MMP-9 and PAI-1, all biomarkers were positively associated with age. Desmosines and IL-8 were significantly higher in women compared to men. Current smokers had significantly higher levels of CRP, PAI-1, TIMP-1 and HGF, and significantly lower CC16, after adjustments for age, sex, BMI and height.

Relationships between biomarkers and lung function measures

The relationships between biomarkers and measures of lung function are presented in Table 3. After adjustments for age, sex, height, smoking and BMI, desmosines were significantly and inversely associated with FEV_1 and D_LCO. CC16 was significantly associated with RV/TLC (inversely). CRP was inversely associated with FVC and D_LCO. PAI-1

Table 1

Table 1 Lung function, smoking status and levels of biomarkers in the study cohort.

N	**357**
Age (years)	59 ± 8.5
Men (%)	37
Smoking status (%)	
Current smokers	20
Ex-smokers	42
Occasional smokers	5.9
Never smokers	32
Lung function	
FEV_1 (L) (men)	3.2 ± 0.72
FEV_1 (L) (women)	2.4 ± 0.52
FVC (L) (men)	4.6 ± 0.80
FVC (L) (women)	3.3 ± 0.62
D_LCO (mL/min/mmHg) (men)	28 ± 6.3
D_LCO (mL/min/mmHg) (women)	20 ± 4.7
RV/TLC (men)	0.37 ± 0.08
RV/TLC (women)	0.39 ± 0.07
GOLD stage (%)	
Normal	67
GOLD I	20
GOLD II	12
GOLD III	1.4
Biomarkers	
Desmosines, nmol/L ($n = 326$)	0.47 (0.16–1.4)
SPD, ng/mL ($n = 298$)	66 (8.6–280)
MMP-9, ng/mL ($n = 279$)	55 (15–330)
IL-8, pg/mL ($n = 303$)	4.9 (0.82–70)
HGF, pg/mL ($n = 246$)	120 (57–320)
PAI-1 concentration, ng/mL ($n = 323$)	2.4 (0.31–15)
CRP, mg/L ($n = 347$)	1.6 (0–39)
CC16, ng/mL ($n = 293$)	4.6 (1.0–15)
TIMP-1, pg/mL ($n = 299$)	150 (2.1–320)
PAI-1 activity, U/ml ($n = 304$)	3.5 (1.6–80)

Values are median (range), mean ± standard deviation or %.

concentrations were significantly associated to RV/TLC (positively), and TIMP-1 was negatively associated to D_LCO.

The study sample was also divided into quartiles based on the biomarker concentrations, and the lung function measures were compared across the quartiles in a general linear model (Supplement Table 1). The results from this analysis were consistent with the findings from the multiple linear regression models.

Biomarkers and lung function in subjects with COPD

The relationships between biomarkers and lung function were also analyzed in subjects with COPD, defined as $FEV_1/FVC < 0.70$. The numbers with COPD ranged between 90 and 110 for the various biomarkers (Table 4). Desmosines were significantly and inversely associated with FEV_1, FVC and D_LCO after adjustments for confounding factors. Desmosines showed no significant relationship with lung function in subjects without COPD, except for a significant relationship between desmosines and D_LCO ($\beta = -0.12$, $p = 0.026$) (data not shown). In subjects with COPD, CRP was inversely associated with FEV_1, FVC, RV/TLC and D_LCO (Table 4). CRP was not significantly associated with lung function in subjects without COPD (data not shown).

MMP-9 was associated with RV/TLC in subjects with COPD ($\beta = 0.20$, $p < 0.05$). No other significant relationship was observed in the subgroup with COPD.

PAI-1 activity

Since a substantial number of subjects had PAI-1 activity below LLOQ, PAI-1 activity was categorized into five groups (Table 5). High PAI-1 activity was associated with BMI, but not significantly with age, sex or smoking. After adjustments for age, sex, BMI, smoking and height, FEV_1 and FVC were significantly lower, and RV/TLC was significantly higher, in subjects with high PAI-1 activity.

There was no significant relationship between PAI-1 activity and lung function when subjects with COPD ($n = 98$) were analyzed separately. However, when comparing the highest vs. lowest categories of PAI-1 activity in subjects without COPD, FEV_1 and FVC were significantly lower and RV/TLC significantly higher after adjustments for confounding factors (not shown).

Table 2 Factors associated with plasma concentrations of nine biomarkers. Results from multiple linear regression analyses.

	Desmosines[a]	SPD[a]	MMP-9[a]	IL-8[a]	HGF[a]	CC16	TIMP−1	PAI-1 concentrations[a]	CRP[a]
N	326	298	279	303	246	293	299	323	347
Age (1 yr)	0.023***	0.012 ***	0.0010	0.014***	0.013***	0.091***	1.4***	0.0046	0.028***
Female (vs male)	0.090*	−0.026	−0.13	0.22*	−0.046	0.10	9.2	0.075	−0.15
Smoking (Yes vs No)	0.055(*)	0.016	−0.010	0.13(*)	0.082*	−1.15***	11*	0.19*	0.35**
BMI (1 kg/m²)	0.018***	−0.013(*)	0.022**	0.014(*)	0.012**	0.0052	2.2***	0.056***	0.13***
Height (1 cm)	0.0017	−0.001	−0.0077	0.0051	0.0025	0.016	1.1**	0.0087	−0.0013

(*) $p < 0.1$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.
Values are beta-coefficients from a multiple linear regression.
[a] Log transformed values were used in the analysis.

Table 3 Relationships between concentrations of plasma biomarker and measures of lung function. Results from multiple linear regression analyses.

	Desmosine[a]	SPD[a]	MMP-9[a]	IL-8[a]	HGF[a]	CC16	TIMP−1	PAI-1 concentration[a]	CRP[a]
N	326	298	279	303	246	293	299	323	347
FEV_1	−0.10*	−0.051	−0.046	−0.038	−0.042	0.068(*)	−0.040	−0.051	−0.069(*)
FVC	−0.072(*)	−0.030	0.019	−0.024	−0.021	0.018	−0.040	−0.031	−0.071*
RV/TLC	0.098	0.033	0.061	0.046	0.029	−0.14*	0.061	0.10*	0.013
D_LCO	−0.21***	0.001	−0.001	−0.035	−0.087(*)	−0.019	−0.099*	−0.026	−0.11**

Values are standardized beta-coefficients, adjusted for age, gender, height, BMI and smoking.
(*) $p < 0.1$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.
[a] Log transformed values were used in the analysis.

Comparisons within twin pairs

Correlations of the differences within twin pair of same gender are presented in Supplementary Table 2. Overall, there were few significant correlations when intra pair differences were studied. However, ΔSPD showed significant correlations with $ΔD_LCO$ ($r = −0.35$, $p < 0.001$) and ΔFVC ($r = −0.23$, $p = 0.03$). These correlations remained significant when the analysis was restricted to 70 pairs with concordant smoking status. Significant correlations were also observed between ΔCC16 and $ΔFEV_1$, and between ΔIL8 and $ΔD_LCO$.

Discussion

The present study investigated the relationships between a panel of blood biomarkers and physiological measures of lung function in a population-based sample of twins. CRP, desmosines, TIMP-1, CC16, PAI-1 activity and PAI-1 concentration were all associated with at least one physiological measure of lung function. However, when subjects with COPD were analyzed separately, two biomarkers, i.e., CRP and desmosines, showed substantial correlations with several measures of lung function. Except for a significant association between MMP-9 and RV/TLC, no relationship was found for any of the other biomarkers in subjects with COPD.

Several previous studies have explored the relationships between different biomarkers and reduced lung function or COPD, and the results have varied a lot between studies. In a recent report from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study,[17] FEV_1 at baseline was inversely associated with fibrinogen and CRP, and positively associated with CC16 and IL-8. CC16 was also associated with change in FEV_1 during follow-up. SPD showed no significant association with FEV_1.[17] In a proteomics study, a large number of biomarkers, including MMP-9, TIMP-1 and IL-8, were significantly increased in COPD patients compared to controls.[18] In contrast, lower TIMP-1 and higher IL-8 in COPD patients were reported by Shaker et al.[7] Increased CRP and IL-8 concentrations were associated with COPD status in a case-control study of COPD patients and 'reference subjects', but no significant association was found for IL-6 or fibrinogen.[6] In a study of COPD patients, CRP and MMP-9 were associated with rapid FEV_1 decline, but no significant relationship was observed for IL-8, TIMP-1, IL-6 or fibrinogen.[5] It is conceivable that inconsistent findings in part could be due to differences between study populations, study designs and degree of control for confounding factors. Many biomarkers are strongly correlated to confounding factors, such as age, sex, smoking and BMI (Table 2), which illustrates the importance of adequate control for these factors in studies of COPD and lung function.

Desmosines and CRP were associated with lung function in the subgroup with COPD, while no relationships were found in the group without COPD. In contrast, PAI-1 activity was associated with lung function in the whole sample and

Table 4 Relationships between concentrations of plasma biomarker and measures of lung function in subjects with COPD ($FEV_1/FVC < 0.70$). Results from multiple linear regression analyses.

	Desmosines[a]	SPD[a]	MMP-9[a]	IL-8[a]	HGF[a]	CC16	TIMP−1	PAI-1 concentration[a]	CRP[a]
N (Men/women)	46/64	44/58	39/54	44/59	40/50	42/53	41/58	45/64	46/64
FEV_1	−0.22*	−0.089	−0.087	−0.010	0.0071	−0.047	0.0004	−0.019	−0.26**
FVC	−0.19*	−0.080	−0.064	0.024	−0.0015	−0.029	0.016	0.017	−0.27***
RV/TLC	0.18	0.090	0.20*	−0.021	0.074	−0.16	0.039	0.091	0.28**
D_LCO	−0.30***	−0.036	0.033	−0.019	−0.16	−0.023	−0.102	0.053	−0.23**

Values are standardized beta-coefficients, adjusted for age, gender, height, BMI and smoking.
(*) $p < 0.1$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.
[a] Log transformed values were used in the analysis.

Table 5 Relationships between PAI-1 activity and measures of lung function.

	PAI-1 activity					p between groups (4 df)
	<3.1 U/ml	3.1—4.3 U/ml	4.4—6.0 U/ml	6.0—11 U/ml	11—80 U/ml	
N	137	41	42	42	42	
Men (%)	29	51	38	45	40	0.077
Age, years (mean $\pm$ SD)	59 $\pm$ 9.0	57 $\pm$ 8.0	60 $\pm$ 8.6	59 $\pm$ 7.2	60 $\pm$ 7.8	0.66
BMI, kg/m^2 (mean $\pm$ SD)	24 $\pm$ 3.0	25 $\pm$ 4.0	26 $\pm$ 3.1	26 $\pm$ 3.9	28 $\pm$ 4.2	<0.001
Smoking (yes) %	25	27	17	31	35	0.35
Lung function						
FEV_1 (L)	2.8 + 0.04	2.7 + 0.07	2.8 + 0.07	2.9 + 0.07	2.6 + 0.07 ($p = 0.003$)	0.007
FVC (L)	3.9 + 0.05	3.8 + 0.08	3.8 + 0.08	4.0 + 0.08	3.6 + 0.08 ($p = 0.01$)	0.024
RV/TLC	0.37 + 0.005	0.38 + 0.009	0.38 + 0.009	0.36 + 0.009	0.40 + 0.10 ($p = 0.009$)	0.023
D_LCO (mL/min/mmHg)	23 + 0.38	22 + 0.68	24 + 0.67	23 + 0.67	22 + 0.69	0.15

Lung function measures are presented as mean values + standard error adjusted for age, sex, height, BMI and smoking in a general linear model.
p-values in brackets are compared to reference category <3.1 U/ml.

in those without COPD, but no significant relationship was found in the COPD group. CRP, desmosines and PAI-1 activity are assumed to reflect different aspects of the underlying disease process. It could be speculated that CRP and desmosines could reflect disease activity in established COPD, while other biomarkers may indicate disease processes at an early stage or other phenotypes with reduced lung function.

Of the biomarkers in this study, CRP has been studied most frequently and was often associated with COPD.[2,4] CRP is generally considered a non-specific marker of systemic inflammation, but it has also been proposed that CRP could be causally related to the disease process in COPD.[19] However, two large studies of COPD or lung function using the 'Mendelian randomization' approach suggest that increased CRP concentrations *per se* have no causal role.[20,21] It therefore seems likely that CRP is an unspecific risk marker and not a causal risk factor. The relationships between CRP and lung function could be explained by reverse causation or 'systemic spill over' from lungs to systemic circulation. Unmeasured confounding factors, which may include other molecules in the inflammatory cascade, is another possible explanation for the relationship between CRP and lung function. Similar studies of the MMP-9 gene have shown inconsistent results with respect to polymorphisms in the MMP-9 gene and COPD.[22,23]

There are few previous studies of plasma desmosines in relation to lung function.[10,24,25] It was reported that desmosines were higher in COPD patients than in healthy subjects,[24,25] and we recently reported that desmosines in plasma as well as urine are associated with lung function measures.[10] Desmosines are cross-linking structures of the elastin fibers, which are released as a result of elastin degradation. Matrix degradation is an important feature in COPD and emphysema and biomarkers that reflect this process could be important for early detection and monitoring of disease activity. Even though much elastin is

located in the lungs, it is important to note that desmosines cannot be considered lung-specific markers, since desmosines also originate from elastin in, e.g., skin and large blood vessels.

Besides its role as an inhibitor of fibrinolysis, PAI-1 has also been associated with obesity, metabolic syndrome, atherosclerosis and inflammation.[13] Increased PAI-1 concentrations were reported in plasma and sputum from COPD patients compared to controls.[14] A recent experimental study reported that PAI-1 stimulates inflammation and migration of inflammatory cells in a culture of alveolar epithelial cells.[26] In the present study, PAI-1 activity showed substantial correlations with lung function in subjects without COPD, but not in those with FEV_1/FVC < 0.70. This suggests that PAI-1 activity could reflect early signs of declining lung function. Even though we adjusted for BMI, it should be noted that we were unable to control for all components of the metabolic syndrome. For example, diabetes and insulin resistance are factors associated with low lung function as well as high PAI-1 values.[13,27,28]

It is well known that concentrations of biomarkers could differ between studies because of differences between laboratory methods. In this study, the mean PAI-1 activity was low and many individuals had values below LLOQ. Therefore, PAI-1 activity was categorized in the analysis. However, the Spearman correlation between PAI-1 activity and PAI-1 concentration was $r = 0.79$ and, as expected, PAI-1 activity was strongly associated with BMI. Therefore, we have no reason to question the validity of the PAI-1 activity, despite the low mean values in this study.

In contrast to the results for the entire cohort of twins, the within-pair analysis showed no significant correlation between lung function and desmosines or CRP, respectively (Supplement Table 2). These correlations were based on approximately 100 twin pairs of same gender and the statistical power was therefore substantially lower. There

were, however, significant correlations between within pair differences in SPD and lung function measures, which was in contrast to the results for the entire cohort. Overmatching is a concern when comparing differences within twin pairs, especially for factors with a strong genetic component. Since lung function and CRP both are genetically determined, this could be a possible explanation.[16,29] An alternative explanation could be that confounding remains in the analysis of the entire cohort, despite multivariate adjustment for major determinants of low lung function. Although we find this explanation unlikely, residual confounding can never be completely excluded.

This study was performed in a sample from the population-based twin registry in Sweden. The lung function impairment was therefore mild in most cases, and only five subjects were in stage 3 of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification.[30] The relationships between lung function and biomarkers could be different in a group of patients with clinically significant COPD. The use of twins for the study could also have influenced the outcome as the selection procedure may have resulted in an over-representation of certain groups, or because genetic factors may influence the relationships studied. However, we have not found any inherent problems in the data that would invalidate the results. Longitudinal studies are needed to address whether the biomarkers are associated with a declining lung function and disease progression.

In conclusion, several relationships with lung function could be found among the blood markers in this panel of proposed biomarkers for COPD. When subjects with COPD were analyzed separately, CRP and desmosines showed substantial correlations with several measures of lung function. These biomarkers are assumed to reflect systemic inflammation and elastin degradation.It is suggested that CRP and desmosines could be useful markers of disease severity in COPD.

Conflicts of interest

The study was conducted at AstraZeneca R&D. GE, CL, MGV, UN and KFS are current or former employees at AstraZeneca R&D.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rmed.2012.05.004.

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