Eur Respir J 2009; 34: 95–102
DOI: 10.1183/09031936.00156508

Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD

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ABSTRACT: Surfactant protein (SP)-D is a lung-derived protein that has been proposed as a biomarker for inflammatory lung disease.

Serum SP-D was evaluated as a biomarker for components of chronic obstructive pulmonary disease (COPD) in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) cohort and its response assessed to the administration of the anti-inflammatory agent prednisolone.

The median level of serum SP-D was significantly elevated in 1,888 individuals with COPD compared to 296 current and former smokers without airflow obstruction (121.1 and 114.3 ng·mL^{-1}, respectively; $p=0.021$) and 201 nonsmokers (82.2 ng·ml^{-1}; $p<0.001$). There was no correlation with the severity of COPD. Individuals with COPD who had a serum SP-D concentration that was greater than the 95th percentile of nonsmokers (175.4 ng·mL^{-1}) showed an increased risk of exacerbations over the following 12 months (adjusted OR 1.30; 95% CI 1.03–1.63). Treatment with 20 mg·day^{-1} prednisolone for 4 weeks resulted in a fall in serum SP-D levels (126.0 to 82.1 ng·mL^{-1}; $p<0.001$) but no significant change in post-bronchodilator forced expiratory volume in 1 s.

Serum SP-D concentration is raised in smokers and may be useful in identifying individuals who are at increased risk of exacerbations of COPD. It may represent an intermediate measure for the development of novel anti-inflammatory agents.

KEYWORDS: Biomarker, bronchitis, emphysema, exacerbation, inflammation, prednisolone

Chronic obstructive pulmonary disease (COPD) is a multicomponent condition that is characterised by airways obstruction, emphysema, mucus hypersecretion and systemic disease that vary in proportion between affected individuals [1, 2]. The development of disease is intimately associated with the inhalation of noxious agents and, in particular, cigarette smoke [3]. There is clearly an urgent need for a simple biomarker that can be used in the diagnosis of COPD and to assess prognosis and the effectiveness of therapeutic interventions. Biomarkers have been assessed in urine, blood, sputum, bronchoalveolar lavage fluid, skin and exhaled breath condensate, but none is widely accepted to be reproducible and to discriminate between smokers with and without airflow obstruction [4]. Moreover, none has proved useful as a robust end-point in clinical trials.

Surfactant protein (SP)-D is a large hydrophilic protein that is a member of the collagen-containing C-type lectins or collectins [5]. Its structure is based on a triple-helical collagen region and a C-terminal homotrimeric lectin or carbohydrate recognition domain. Four of the homotrimeric subunits of SP-D are assembled *via* their N-terminal region into a 520-kDa dodecameric structure that can further oligomerise to form multimers. SP-D is found in the endoplasmic reticulum of type II pneumocytes and the secretory granules of Clara cells [6]. It makes an important contribution to surfactant homeostasis and pulmonary immunity [5]. SP-D plays a role in: protecting against viral infection; clearance of bacteria, fungi and apoptotic cells; and resolution of inflammation [7]. Mice that lack SP-D develop chronic inflammation and emphysema that can be prevented by administration of truncated recombinant human SP-D [8]. Since SP-D is synthesised

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Received:
Oct 15 2008
Accepted after revision:
Jan 02 2009
First published online:
Jan 22 2009

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

predominantly within the respiratory tract, it has been evaluated as a potential biomarker in small numbers of individuals with community-acquired pneumonia [9], drug-induced lung disease [10, 11], interstitial fibrosis [12] and allergic bronchopulmonary aspergillosis in cystic fibrosis [13]. Levels are reduced in bronchoalveolar lavage fluid from individuals with COPD [14], and there was a weak inverse relationship between serum SP-D level and forced expiratory volume in 1 s (FEV_1) in 23 individuals with advanced COPD [15]. The utility of serum SP-D as a biomarker for components of the COPD phenotype was evaluated and the effect assessed of oral corticosteroids on levels of this biomarker.

MATERIALS AND METHODS

The population under study was the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE; study identification number SCO104960; Clinicaltrials.gov identifier NCT00292552) cohort.

The aims and operational aspects of the ECLIPSE cohort have been described elsewhere [16, 17]. Briefly, the ECLIPSE study is a 3-yr multicentre longitudinal observational study to identify novel end-points in COPD. Individuals aged 40–75 yrs were recruited to the study if they had a smoking history of $\geqslant$10 pack-yrs, a post-bronchodilator FEV_1/forced vital capacity (FVC) ratio of $\leqslant$0.7 and Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II (FEV_1 50–80% of the predicted value), III (FEV_1 30–50% pred) or IV (FEV_1 <30% pred) COPD [3]. Smoking ($\geqslant$10 pack-yrs) and nonsmoking (<1 pack-yr) control subjects were enrolled if they were aged 40–75 yrs and exhibited normal lung function (post-bronchodilator FEV_1 of >85% pred and FEV_1/FVC of >0.7). Individuals recruited to the study were genotyped for α_1-antitrypsin deficiency. Six PiZZ and 11 PiSZ individuals were identified and excluded from the analysis.

All subjects underwent standardised spirometry following 180 μg (2 puffs) salbutamol [18], with reversible airflow obstruction being defined as an increase in FEV_1 of 15% and $\geqslant$200 mL. All subjects were offered a low-dose computed tomography (CT) scan of the chest in order to exclude non-COPD-related disease and evaluate the severity and distribution of emphysema. The CT scans were evaluated at the central imaging unit of the University of British Columbia (Vancouver, BC, Canada). The extent of emphysema was assessed in two ways. First, it was independently scored by two radiologists who were blind to the individual's lung function. Emphysema was reported as trivial, mild, moderate, severe and very severe if it affected <5, 5–25, 25–50, 50–75 and >75% of the lungs, respectively. A consensus reading was obtained when there was a difference of more than one emphysema category between the two observers. Otherwise, the mean of the two readings was used in the analysis. Second, emphysema was assessed by the percentage of the lung with attenuation of <-950 HU using Pulmonary Workstation 2.0 software (VIDA Diagnostics, Inc., Iowa City, IA, USA).

Assessment of exacerbations in the ECLIPSE cohort

COPD subjects were asked about exacerbations 3, 6 and 12 months after enrolment in the study. In addition, they were contacted by telephone every month by the study staff and asked about details of exacerbations during the previous month. Specifically, subjects were asked whether they had been unwell in the last month, whether they had seen a doctor or been to hospital and whether they had taken any medication for exacerbations (oral corticosteroids or antibiotics). The data were analysed 12 months after enrolment into the study.

Effect of oral corticosteroids on serum surfactant protein D in individuals with chronic obstructive pulmonary disease

The effects of oral prednisolone were investigated in a separate study (Clinicaltrials.gov identifier NCT00379730).

Current/former smokers aged 40–80 yrs with a post-salbutamol FEV_1 of 30–80% pred and chronic bronchitis (n=89) were recruited to a study that was separate from the ECLIPSE study. Chronic bronchitis was defined as daily cough productive of sputum for 3 months for 2 yrs successively [19]. Individuals were excluded if they had had an exacerbation of COPD requiring steroid or antibiotics in the month prior to the 28-day screening period or were taking oral or inhaled steroids for >14 days consecutively in the 6 months prior to screening. All subjects were offered a CT scan of the chest, which was performed and analysed as detailed for the ECLIPSE study. Individuals were randomised to receive either placebo or 20 $mg \cdot day^{-1}$ prednisolone for 4 weeks, 10 $mg \cdot day^{-1}$ prednisolone for 1 week and 5 $mg \cdot day^{-1}$ prednisolone for 1 week. Both groups were followed-up for 2 weeks following cessation of treatment. Serum samples were taken at baseline and every 2 weeks throughout the study.

Measurement of serum surfactant protein D

Whole blood was collected into vacutainer tubes at the start of the studies. Serum was prepared by centrifugation for 10–15 min at $1,500 \times g$. The serum was collected and stored at -80°C until analysed. Serum SP-D was measured by operators who were blind to an individual's lung disease or treatment group using a colorimetric sandwich immunoassay method (BioVendor, Heidelberg, Germany) according to the manufacturer's instructions. Samples were routinely tested at 5-fold dilution with the dilution buffer supplied by the manufacturer. Samples with out-of-range results were retested at higher dilution. The concentration of SP-D in the diluted samples was interpolated from the standard curve of recombinant human SP-D (molecular mass 41 kDa) and then corrected for the dilution factor. The assay had a validated range of 1.56–100 $ng \cdot mL^{-1}$, with an intra-assay coefficient of variation and relative error of 1.98–4.06% and -7.32– -1.40%, respectively, and an inter-assay coefficient of variation and relative error of 4.80–5.84% and -12.22– -2.46%, respectively.

Statistical analysis

The reproducibility of SP-D measurement in the ECLIPSE cohort was assessed through Bland–Altman plots [20]. Owing to the non-normality of SP-D values identified by Shapiro–Wilk and Kolmogorov–Smirnov tests, all SP-D measurements in the ECLIPSE cohort were logarithmically transformed prior to analysis. All comparisons between subject groups were then conducted by ANOVA based on the transformed values. Spearman's correlation coefficients (based on ranks) were calculated for correlations between SP-D level and clinical parameters. In the prednisolone study, the effect of prednisolone on serum SP-D levels and FEV_1 was analysed by

ANCOVA, adjusting for baseline value and study site. In both studies, ANOVA and Cochran–Mantel–Haenszel tests were used to compare subject groups. SAS® Version 8.2 (SAS Institute, Inc., Cary, NC, USA) was used to carry out all analyses.

Ethics

The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and were approved by the relevant ethics and institutional review boards at the participating centres.

RESULTS

Assessment of surfactant protein D in the ECLIPSE cohort

Serum SP-D was measured in 1,888 individuals with COPD from the ECLIPSE cohort, 296 smoking controls with no airflow obstruction and 201 nonsmoking controls (table 1; fig. 1a). Median levels of serum SP-D were higher in the mixture of current and former smokers with COPD compared to those without COPD (121.1 and 114.3 ng·mL^{-1}, respectively; p=0.021), and in smokers compared to nonsmokers with no airflow obstruction (114.3 and 82.2 ng·mL^{-1}, respectively; p<0.001). The serum SP-D concentration was similar in males and females who were nonsmokers (80.8 and 83.3 ng·mL^{-1}; n=74 and 127, respectively), smoker controls (118.3 and 109.5 ng·mL^{-1}; n=161 and 135, respectively; p=0.732) and those with COPD (123.3 and 117.0 ng·mL^{-1}; n=1,222 and 666, respectively; p=0.081). Serum SP-D levels were not associated with COPD disease severity as defined by GOLD status. There was no difference in serum SP-D level in individuals with COPD or in smoker controls who reported chronic bronchitis compared with those who did not have this symptom. Moreover, there was no correlation between serum SP-D level and either the radiologists' emphysema score or areas of low attenuation on the CT scan (<-950 HU). There were weak correlations between serum SP-D level and age (r=0.11; p<0.001) and body mass index (BMI) (r=-0.13; p<0.001).

Twin studies have shown that serum levels of SP-D are elevated by smoking [21]. Levels of SP-D were, therefore, analysed in groups divided into current and former smokers (fig. 1b). Serum levels of SP-D were higher in current than in former smoker controls, but were significantly higher in both current and former smokers diagnosed with COPD (p=0.024 and 0.001, respectively). The effect of smoking was apparent across all severities of COPD, as defined by GOLD status.

Serum surfactant protein D and risk of exacerbations of chronic obstructive pulmonary disease

Data were available for 2,189 (92%) of the 2,385 individuals after 12 months of follow-up. There were 2,351 exacerbations (1,446 and 905 in former and current smokers, respectively), as defined by episodes of worsening symptoms that were self-managed by the subject. These were reported by 1,093 individuals with COPD (670 and 423 former and current smokers, respectively; the range of exacerbations in any individual was 1–11). There was no effect of current smoking on the incidence of exacerbations (56.9 and 58.7% in current and former smokers, respectively) and a weak negative correlation between the incidence of exacerbations and percentage predicted FEV1 (r=-0.15; p<0.001). Baseline serum SP-D level was not associated with either decline in FEV1 (r=0.004; p=0.866), percentage predicted FEV1 (r=0.004; p=0.837) or FVC (r=-0.033; p=0.117). The median serum SP-D concentration was similar between those individuals who had had one or more exacerbations during 12 months of follow-up and those who had had no exacerbations (121.5 *versus* 120.3 ng·mL^{-1}; n=1,093 and 795, respectively; p=0.062). Moreover, there was no correlation between serum SP-D level and the number of exacerbations reported during the 12-month follow-up or whether an individual required hospitalisation for an exacerbation. Although only a small number of individuals died during the first 12 months of the ECLIPSE study, there was a trend towards higher baseline median SP-D concentrations in those individuals who died during follow-up

TABLE 1 Assessment of serum surfactant protein (SP)-D in individuals with and without chronic obstructive pulmonary disease (COPD)

	COPD subjects	Smoker controls	Nonsmoker controls	p-value#
Subjects n	1888	296	201	
Age yrs	63.4±7.2	54.7±8.9	53.2±8.6	<0.001
Males n (%)	1222 (65)	161 (54)	74 (37)	0.001
Smoking history pack-yrs	49.2±27.3	32.0±22.1	0.4±0.5	<0.001
Current smoker n (%)	746 (40)	201 (68)	0 (0)	<0.001
FEV1 L	1.4±0.5	3.4±0.8	3.3±0.8	<0.001
FEV1 % pred	48.7±15.5	108.6±12.1	114.8±14.0	<0.001
FEV1/FVC	0.45±0.11	0.79±0.05	0.81±0.05	<0.001
CT scans n	1496	260	165	
Low attenuation area¶ %	16.9±11.8	2.2±2.9	3.9±4.0	<0.001
SP-D ng·mL^{-1}	121.1 (84.9–174.2)	114.3 (75.6–162.3)	82.2 (56.1–117.7)	0.021

Data are presented as mean±SD or median (interquartile range) unless otherwise indicated. The lung function measurements followed the administration of 180 μg salbutamol. The number of computed tomography (CT) scans is the number of scans available for qualitative analysis to assess the percentage of the lungs with a density of <-950 HU. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; % pred: percentage of the predicted value. #: COPD subjects *versus* smoker controls (p<0.001 for COPD subjects *versus* nonsmoker controls for all parameters); ¶: <-950 HU.

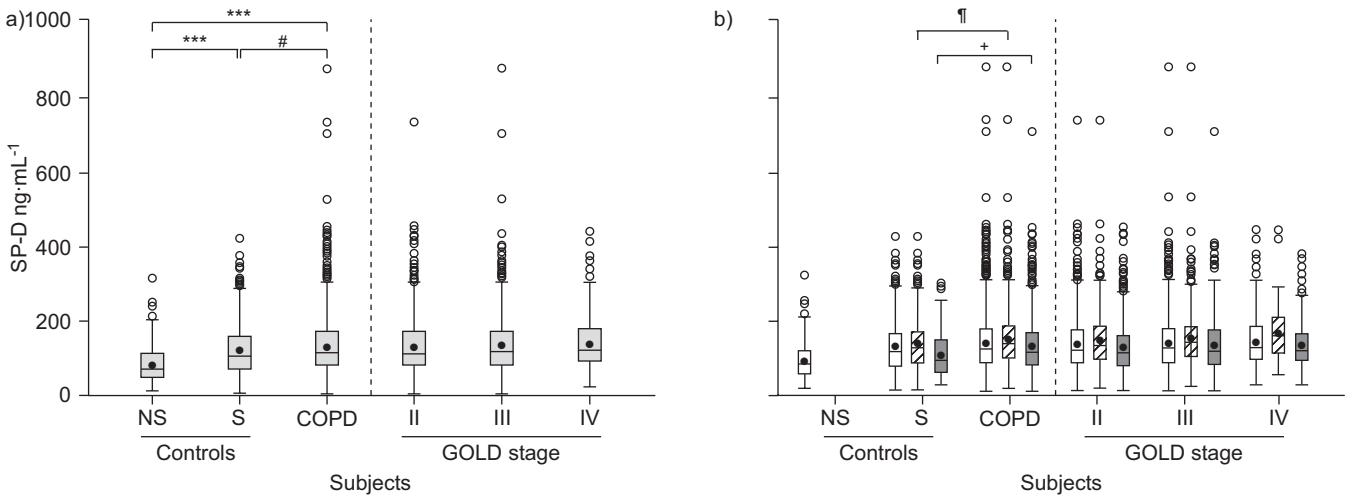

FIGURE 1. a) Serum surfactant protein (SP)-D levels in individuals with chronic obstructive pulmonary disease (COPD) and controls; and b) effect of current smoking on SP-D levels in smoking controls and individuals with COPD (shown as a whole and divided into groups based on Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification). Vertical bars represent median and interquartile range (●: mean; ○: outliers). The number of individuals (□)/current smokers (▨) in each group was: nonsmoker (NS) controls 201/0, smoker (S) controls 296/201, all COPD subjects 1,888/746, GOLD stage II COPD 846/334, GOLD stage III COPD 811/324, and GOLD stage IV COPD 229/76 (■: former smokers). ***: $p<0.001$; #: $p=0.021$; ¶: $p=0.024$; +: $p=0.001$.

compared to those who remained alive (138.8 and 120.9 ng·mL^{-1}; n=40 and 1,848, respectively; p=0.125).

Serum SP-D concentration was assessed as a continuous variable in a multivariate model for its ability to predict the occurrence of at least one exacerbation during the 12-month follow-up. The results showed an OR of 1.22 (95% CI 1.07–1.39) for exacerbations for each 100 ng·mL^{-1} increase in SP-D level after adjusting for sex, percentage predicted FEV1, reversibility and those individuals taking inhaled corticosteroids. This was more marked if the analysis was restricted to those individuals with a baseline SP-D level in the upper quartile (OR 1.42; 95% CI 1.02–1.97). The effect may result from a small number of very high outliers. However, the findings were even more marked if the outliers with SP-D concentrations above the 99th percentile (382.7 ng·mL^{-1}) were excluded (OR 1.58; 95% CI 1.02–2.44). These results may simply reflect those individuals who reported exacerbations prior to enrolment in the study. The analysis was, therefore, repeated in the subset of individuals who did not report any exacerbation during the year prior to enrolment in the study. Serum SP-D concentration remained associated with an increased risk of exacerbations (OR 1.23; 95% CI 1.02–1.49). The results were unchanged if the analysis was repeated with either diuretic or β-blocker medication being included as confounding factors.

The 95th percentile of serum SP-D level in the nonsmokers was 175.5 ng·mL^{-1}. This concentration was used to categorise subjects with COPD as having either high or low levels of serum SP-D. The OR for exacerbations of COPD was 1.30 (95% CI 1.03–1.63) in individuals with high serum levels of SP-D after adjusting for sex, percentage predicted FEV1 and those individuals taking inhaled corticosteroids (neither age, smoking status, smoking history in pack-years nor reversibility were significant predictors in this model). Repeating this analysis with the 75th percentile of serum SP-D concentration for COPD subjects (174.2 ng·mL^{-1}) as the cut-off for categorising the subjects with COPD gave similar results (OR 1.28; 95% CI 1.02–1.61). Similar results were also obtained if exacerbations were defined based on the requirement for antibiotics (~80% of all exacerbations; OR 1.31; 95% CI 1.05–1.64).

Assessment of the reproducibility of serum surfactant protein D

It is important to know whether serum SP-D level is a reproducible biomarker, and so it was measured in an age-matched subgroup of 195 individuals with COPD, 36 smoker controls and 36 nonsmoking controls selected from the ECLIPSE cohort (table 2). The individuals with COPD and smoker controls were all former smokers in order to reduce the variability associated with smoking status. SP-D levels were reproducible in nonsmokers, former smokers without airflow obstruction and across all severities of COPD when measured over a period of 3 months (coefficient of repeatability 70.20 ng·mL^{-1}; variability 26%) (fig. 2; table 2).

Effect of prednisolone on serum surfactant protein D in individuals with chronic obstructive pulmonary disease

Serum levels of SP-D were similar in the ECLIPSE study regardless of background therapy, including inhaled corticosteroids and long-acting β_2-agonists. However, a modest fall in serum SP-D concentration has been reported in individuals with COPD following treatment with inhaled corticosteroids, suggesting that SP-D may be a biomarker for anti-inflammatory therapy [22]. The effect of administration of a systemic corticosteroid on serum SP-D level was tested in a study separate from the ECLIPSE study. A total of 89 current or former smokers diagnosed with chronic bronchitis and COPD were recruited and randomised to receive either oral prednisolone or placebo. The groups were well matched for age, sex, lung function, degree of reversibility and smoking history in pack-years (table 3). There were five withdrawals in the prednisolone group and four in the placebo group during the course of the study. Treatment with prednisolone resulted in a

TABLE 2 Assessment of the reproducibility of serum surfactant protein (SP)-D measurement

	COPD subjects	Smoker controls	Nonsmoker controls	p-value#
Subjects n	195	36	36	
Age yrs	64.5±6.0	60.8±7.7	59.7±8.8	0.002
Males n (%)	141 (72)	24 (67)	14 (39)	0.492
Smoking history pack-yrs	45.8±27.2	29.8±16.5	1.0±0.0	0.001
FEV1 L	1.2±0.5	3.2±0.6	3.1±0.7	<0.001
FEV1 % pred	43.9±16.9	108.9±11.8	115.8±12.0	<0.001
FEV1/FVC	0.40±0.12	0.80±0.06	0.80±0.05	<0.001
CT scans n	178	29	32	
Low attenuation area¶ %	22.6±13.5	4.5±4.4	5.4±5.5	<0.001
Baseline SP-D ng·mL^{-1}	103.6 (77.6–143.9)	96.5 (68.6–121.4)	71.8 (49.1–99.8)	0.116
SP-D results at 3 months n	181	35	34	
SP-D at 3 months ng·mL^{-1}	105.1 (70.5–145.2)	95.5 (59.4–120.0)	77.5 (49.5–96.6)	0.232

Data are presented as mean±SD or median (interquartile range) unless otherwise indicated. The lung function measurements followed the administration of 180 μg salbutamol. All of the smoking controls and individuals with chronic obstructive pulmonary disease (COPD) were former smokers. The number of computed tomography (CT) scans is the number of scans available for qualitative analysis to assess the percentage of the lungs with a density of <-950 HU. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; % pred: percentage of the predicted value. #: COPD subjects *versus* smoker controls (p<0.001 for COPD subjects *versus* nonsmoker controls for all parameters); ¶: <-950 HU.

small increase relative to placebo in pre- and post-bronchodilator FEV1 of 97 and 107 mL, respectively (fig. 3a); neither of these changes were significant (p=0.07 and 0.06, respectively). However, treatment with prednisolone resulted in a striking fall in serum SP-D level from 126.0 to 82.1 ng·mL^{-1} at 4 weeks (p<0.001) (fig. 3b). The concentration remained low whilst the subjects were taking 20 mg·day^{-1} prednisolone and rose as the dose of steroids was reduced before returning to baseline 2 weeks after cessation of therapy. There was no change (136.0 ng·mL^{-1} at baseline, 135.8 ng·mL^{-1} at week 4) in serum SP-D level in those individuals who received placebo. The effect of prednisolone was specific for SP-D as there was no significant reduction in serum levels of other inflammatory markers that have been reported to be elevated in COPD (fibrinogen; interleukin-1β, -8 or -6; myeloperoxidase or matrix metalloproteinase-9 (data not shown)).

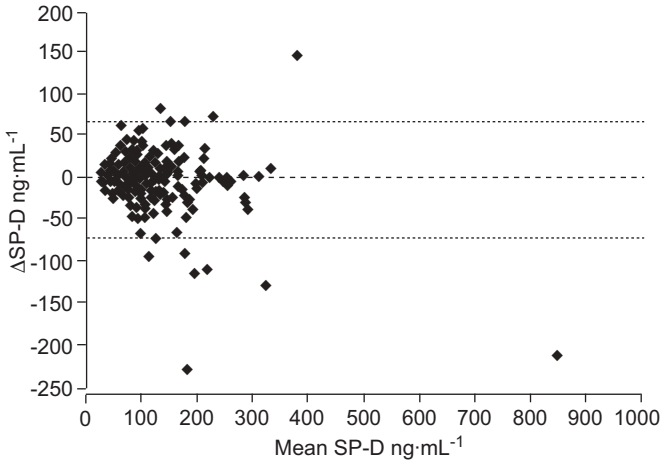

FIGURE 2. Bland–Altman plot for assessment of the reproducibility of serum levels of surfactant protein (SP)-D at baseline and 3 months in 267 individuals with and without chronic obstructive pulmonary disease (--------: no difference (Δ); ·····: 95% limits of agreement). The bias is -2.23 and coefficient of repeatability 70.20 ng·mL^{-1}.

DISCUSSION

The ECLIPSE cohort was used to evaluate serum SP-D as a biomarker for COPD. The median serum SP-D level was significantly higher in current and former smokers with COPD than in those without airflow obstruction. The serum SP-D level was similar in males and females, was unaffected by the presence of chronic bronchitis and did not correlate with either the radiologists' emphysema score or areas of low attenuation on the CT scan (<-950 HU). Moreover, there was no significant increase in serum levels with increasingly severe disease (as assessed by GOLD score). The difference in serum SP-D levels between individuals with COPD and smoker and nonsmoker controls, although significant, is not sufficiently large to use in a screening test to diagnose COPD.

The largest difference in serum SP-D levels occurred between nonsmokers and current/former smokers. Therefore, serum SP-D is a powerful biomarker for smoking. Intrapulmonary SP-D levels rise following the acute exposure of mice to cigarette smoke [23], but are lower in lung lavage fluid from individuals with COPD [14] and cystic fibrosis [24]. Approximately 75% of SP-D is found in bronchoalveolar lavage fluid [5], and it is likely that this hydrophilic protein, or its degradation products, leaks from the lung as a consequence of increased vascular permeability associated with inflammation. It is then detected within the circulation. Thus serum SP-D reflects intrapulmonary inflammation, which would explain the higher levels in smokers with and without COPD.

A normal range for serum SP-D level can be derived from nonsmoking controls. It was striking that those individuals with COPD who had SP-D levels that were greater than the

TABLE 3 Demographics and baseline characteristics of individuals with chronic obstructive pulmonary disease randomised to receive either oral corticosteroids or placebo

	Placebo	Prednisolone
Subjects n	44	45
Age yrs	62.8±8.4	62.6±9.1
Males	32 (73)	35 (78)
FEV1 L	1.35±0.56	1.33±0.56
FEV1 % pred	49.6±15.6	46.6±15.1
FEV1/FVC	0.49±0.12	0.45±0.12
FEV1 reversibility %	15.1±14.7	18.2±17.9
Smoking history pack-yrs	53.4±35.1	48.4±24.5
Current smokers	21 (48)	19 (42)
Salbutamol	35 (80)	36 (80)
Ipratropium bromide	17 (39)	25 (56)
CT scans n	36	40
Emphysema on CT scan	34 (94)	36 (92)

Data are presented as mean±SD or n (%) unless otherwise indicated. Reversibility was defined as an increase in forced expiratory volume in 1 s (FEV_1) following 180 μg salbutamol. The number of individuals with any emphysema on their computed tomography (CT) scan was determined from the radiologists' score. There were five withdrawals in the prednisolone group and four in the placebo group. FVC: forced vital capacity; % pred: percentage of the predicted value.

95th percentile of normal controls showed a greater risk of self-reported exacerbations. These symptoms were reported prospectively and were, therefore, not dependent on recall bias. The findings were unchanged if exacerbations were defined as requiring the administration of antibiotics. Moreover, the risk of exacerbations increased with increasing baseline serum SP-D concentrations, with an even greater risk if the analysis were confined to those individuals in the upper quartile of baseline SP-D level. It is possible that this effect was driven by a few outliers and thus the analysis was repeated following the exclusion of individuals with the highest serum SP-D levels. Again, serum SP-D was associated with exacerbations of COPD. Finally, it is possible that serum SP-D level is affected by comorbid conditions and so the analysis was repeated with either diuretic or β-blocker medication being included as confounding factors. This did not affect the results.

Previous work has shown that plasma C-reactive protein [25] and serum amyloid A [26] are nonspecific markers of exacerbations of COPD, with raised levels of serum amyloid A being associated with more severe episodes [26]. However, serum SP-D is the first biomarker that has been shown to predict an increased risk of exacerbations of COPD in a large prospective cohort. It is perhaps not surprising that individuals with the greatest levels of intrapulmonary inflammation (as evidenced by raised serum SP-D levels) are at the greatest risk of exacerbations since previous studies have shown that the severity of exacerbations of COPD tracks with airway inflammation [27]. Exacerbations of COPD are associated with significant deterioration in health status [28], and thus serum SP-D may be useful in identifying those at greatest risk

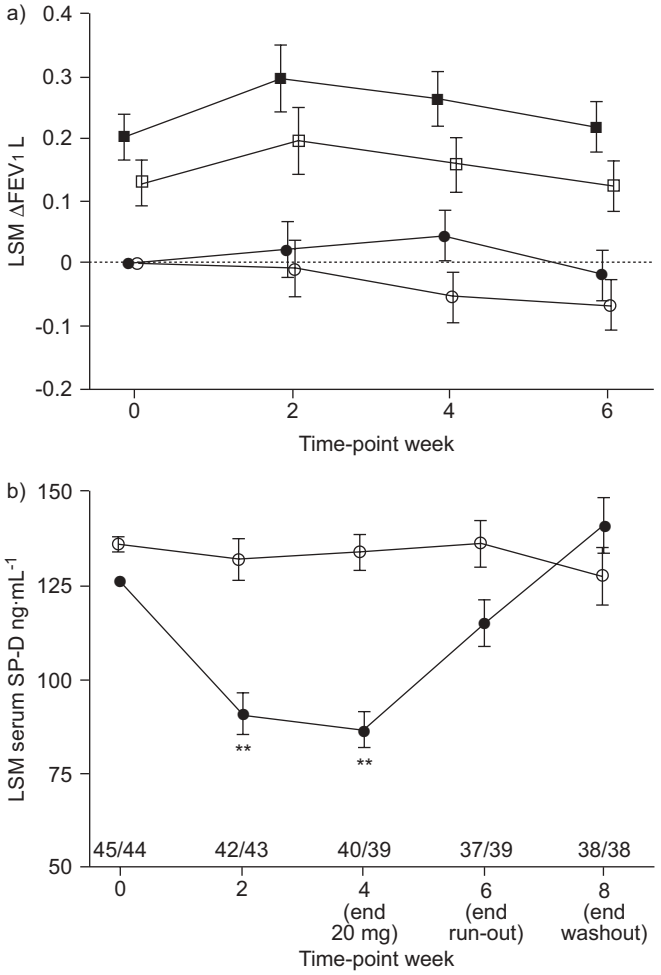

FIGURE 3. Effect of oral corticosteroids in individuals with chronic obstructive pulmonary disease (COPD) on: a) forced expiratory volume in 1 s (FEV_1); and b) serum surfactant protein (SP)-D level. Individuals with COPD were randomised to prednisolone (●; ■) or placebo (○; □). The prednisolone group received 20 mg·day^{-1} prednisolone for 4 weeks, 10 mg·day^{-1} prednisolone for 1 week and 5 mg·day^{-1} prednisolone for 1 week. The effect of prednisolone on FEV_1 is shown before (●; ○) and after (■; □) administration of 180 μg salbutamol. Least square means (LSMs) were adjusted for baseline and study site in the analysis model. The number of individuals at each time-point is shown for prednisolone/placebo in b). Data were analysed by intention to treat and are presented as mean±SEM. Δ: change (from baseline (week 0)). **: $p<0.001$.

and who may, therefore, benefit from treatment with either anti-inflammatory agents or prophylactic antibiotics. If a biomarker is to be used to identify individuals at risk of exacerbations then it must be stable over time. Serum SP-D level was assessed in a different group of individuals in the ECLIPSE cohort. The levels showed 26% variability in nonsmokers and former smokers with and without COPD over a 3-month interval. This variability may be higher in individuals with higher baseline serum SP-D levels.

A biomarker that reflects the intrapulmonary inflammation of COPD should respond to the administration of a potent anti-inflammatory agent such as prednisolone. Indeed, there was a rapid and marked fall in serum SP-D levels whilst individuals

with COPD received oral corticosteroids. Serum SP-D levels returned to baseline following the cessation of treatment. The change in serum SP-D levels occurred in the context of insufficient power to detect a significant change in the standard measures of lung function, FEV_1 and FVC. Prednisolone did not mediate its effects by reducing the expression of SP-D as exogenous steroids increase, rather than reduce, SP-D expression in human lung [29]. It is more likely to be reporting changes in permeability that result from suppression of inflammation. Thus SP-D is exquisitely more sensitive in reporting the changes that result from the administration of oral prednisolone than is lung function. It was difficult to assess the effect of inhaled corticosteroids on serum SP-D level in the present cohort as most subjects were taking this medication.

The association of serum SP-D with COPD reported here arose from a cross-sectional study. It will be important to assess whether SP-D tracks with decline in lung function and progression of emphysema, airways disease and systemic features (such as BMI, fatigue, muscle wasting and systemic inflammation) during the 3 yrs of follow-up of the ECLIPSE cohort. If so, then serum SP-D offers a real prospect of a biomarker that can report disease progression. Other studies are needed to determine whether small molecules that reduce inflammation and suppress SP-D can reduce exacerbations and modify the decline in one or more of the indices that are abnormal in individuals with COPD. If this is the case, then the suppression of serum SP-D levels would provide an intermediate measure of disease modification in COPD.

In summary, a large cohort of individuals with COPD and smoking and nonsmoking controls have been used to show that median serum SP-D levels are elevated, and predict exacerbations, in individuals with COPD. These levels fall following treatment with oral corticosteroids. Thus serum SP-D may be useful as an intermediate measure in the development of anti-inflammatory therapies for COPD.

CLINICAL TRIALS

This study is registered at ClinicalTrials.gov (trial numbers NCT00292552 and NCT00379730).

STATEMENT OF INTEREST

Statements of interest for D.A. Lomas, E.K. Silverman, L.D. Edwards, B.E. Miller, D.H. Horstman and R. Tal-Singer and the study itself can be found at www.erj.ersjournals.com/misc/statements.dtl

ACKNOWLEDGEMENTS

The authors would like to express their thanks to all of the subjects who took part in the present studies. They are grateful to R. Harding (GlaxoSmithKline, London, UK) for help with study NCT00379730 and E. Kurali (GlaxoSmithKline, King of Prussia, PA, USA) for exploratory statistics. The quantitative computed tomography (CT) analysis was performed by the Thoracic Imaging Group at the University of British Columbia (Vancouver, BC, Canada) under the direction of H. Coxson, and the CT scans were qualitatively assessed by N. Müller and P. Nasute Fauerbach.

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