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

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Abstract

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

We have evaluated serum SP-D as a biomarker for components of COPD in the ECLIPSE cohort and have assessed its response to the administration of the anti-inflammatory agent prednisolone.

The median level of serum SP-D was significantly elevated in 1888 individuals with COPD when compared to 296 current and former smokers without airflow obstruction (121.1 and 114.3 ng/mL respectively; $p=0.021$) and 201 non-smokers (82.2 ng/ml; $p<0.001$). There was no correlation with the severity of COPD. Individuals with COPD who had a serum SP-D that was greater than the 95th percentile of non-smokers (175.4 ng/mL) had an increased risk of exacerbations over the following 12 months (adjusted odds ratio 1.30, C.I. 1.03, 1.63). Treatment with 20 mg/day prednisolone for four weeks resulted in a fall in serum SP-D (126.0 to 82.1 ng/mL; $p<0.001$) but no statistically significant change in post-bronchodilator FEV_1.

Serum SP-D 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.

Word count 197

Key words: bronchitis, emphysema, exacerbation, inflammation, prednisolone, biomarker

Introduction

COPD is a multi-component 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, broncho-alveolar lavage, 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 endpoint in clinical trials.

Surfactant protein D (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 dodecamer structure that can further oligomerise to form multimers. SP-D is found in the endoplasmic reticulum of type II pneumocytes and in 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, in the clearance of bacteria, fungi and apoptotic cells and in the 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]. As

SP-D is predominantly synthesized 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 from individuals with COPD [14] and there was a weak inverse relationship between serum SP-D and FEV_1 in 23 individuals with advanced COPD [15]. We have evaluated the utility of serum SP-D as a biomarker for components of the COPD phenotype and have assessed the effect of oral corticosteroids on levels of this biomarker.

Materials and methods

The ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) cohort (SCO104960, Clinicaltrials.gov identifier NCT00292552, appendix 1).

The aims and operational aspects of the ECLIPSE cohort have been described elsewhere [16, 17]. Briefly ECLIPSE is a 3 year multicentre longitudinal observational study to identify novel endpoints in COPD. Individuals aged 40–75 years were recruited to the study if they had a smoking history of ≥10 pack-years, a post-bronchodilator ratio between forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) ≤0.7 and GOLD stage II (FEV_1 50-80% predicted), III (FEV_1 30-50% predicted) or IV (FEV_1 <30% predicted) COPD [3]. Smoking (≥10 pack-years) and non-smoking (<1 pack-year) control subjects were enrolled if they were aged 40–75 years and had normal lung function (post-bronchodilator FEV_1>85% predicted and FEV_1/FVC >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 mcg (2 puffs) of salbutamol [18] with reversible airflow obstruction being defined as an increase in FEV_1 of 15% and at least 200 mL. All subjects were offered a low dose computed tomography (CT) scan of the chest to exclude non-COPD related disease and to evaluate the severity and distribution of emphysema. The CT scans were evaluated at the central imaging unit at the University of British Columbia in Vancouver. The extent of emphysema was assessed in two ways. Firstly 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 1 emphysema category between the 2 observers. Otherwise the average of the 2 readings was used in the analysis. Secondly emphysema was assessed by the percentage of the lung with attenuation below -950 HU using the *Pulmonary Workstation 2.0* software (VIDA Diagnostics, Inc., Iowa City, IA).

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 phone every month by the study staff and asked about details of exacerbations for the previous month. Specifically subjects were asked whether they have been unwell in the last month, if they had seen a doctor or been to hospital and if they have 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 SP-D in individuals with COPD (Clinicaltrials.gov identifier NCT00379730, appendix 2).

Eighty-nine current/former-smokers aged 40 to 80 years with post-salbutamol FEV_1 between 30 and 80% predicted and chronic bronchitis were recruited to a study that was separate from ECLIPSE. Chronic bronchitis was defined as a daily cough productive of sputum for 3 months for 2 successive years [19]. Individuals were excluded if they 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 more than 14 consecutive days in the 6 months prior to screening. All subjects were offered a CT scan of the chest that was performed and analysed as detailed for the ECLIPSE study. Individuals were randomised to receive either placebo or 20 mg/day prednisolone for 4 weeks, 10 mg/day prednisolone for 1 week and 5 mg/day prednisolone for 1 week. Both groups were followed-up for 2 weeks following the cessation of treatment. Serum samples were taken at baseline and every 2 weeks throughout the study.

Measurement of serum SP-D

Whole blood was collected into vacutainer tubes at the start of the study. Serum was prepared by centrifugation at 1500g for 10-15 minutes. The serum was collected and stored at -80 $^{\circ}$C until analyzed. 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 GmbH, 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 a 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 to 100 ng/mL with an intra-assay co-efficient of variation and relative error of 1.98 to 4.06% and -7.32 to -1.40%, respectively and an inter-assay co-efficient of variation and relative error of 4.80 to 5.84% and -12.22 to -2.46%, respectively.

Statistical analysis

Reproducibility of SP-D in the ECLIPSE cohort was assessed through Bland-Altman plots [20]. Due to the non-normality of SP-D values identified by Shapiro-Wilk and Kolmogorov-Smirnov tests, all SP-D values in the ECLIPSE cohort were log-transformed prior to analysis. All comparisons between subject groups were then conducted by analysis of variance (ANOVA) based on the log-transformed values. Spearman correlation coefficients (based on ranks) were calculated for correlations between SP-D and clinical parameters. In the prednisolone study, the effect of prednisolone on serum SP-D and FEV_1 was analysed by analysis of covariance (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 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 relevant ethics and institutional review boards at the participating centres.

Results

Assessment of SP-D in the ECLIPSE cohort

Serum SP-D was measured in 1888 individuals with COPD from the ECLIPSE cohort, 296 smoking controls with no airflow obstruction and 201 non-smoking controls (Table 1 and Fig. 1a). Median levels of serum SP-D were higher in a mixture of current and former smokers with COPD compared to those without COPD (121.1 and 114.3 ng/mL; $p=0.021$) and between smokers and non-smokers with no airflow obstruction (114.3 and 82.2 ng/mL; $p<0.001$). Serum SP-D was similar in men and women who were non-smokers (80.8 and 83.3 ng/mL; n=74 and 127 respectively), smoker controls (118.3 and 109.5 ng/mL; n=161 and 135; $p=0.732$) and those with COPD (123.3 and 117.0 ng/mL; n=1222 and 666; $p=0.081$). Serum SP-D levels were not associated with COPD disease severity as defined by GOLD status. There was no difference in serum level of SP-D 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 and either the radiologists' score of emphysema or areas of low attenuation on the CT scan (<-950 HU). There were weak correlations between serum SP-D and age ($r=0.11$; $p<0.001$) and BMI ($r= -0.13$; $p<0.001$).

Twin studies have shown that serum levels of SP-D are elevated by smoking [21]. The 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 rather than 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 COPD

Data were available for 2189 of the 2385 individuals after 12 months of follow-up (92%). 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; range of exacerbations in any individual 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 FEV1 % predicted ($r=-0.15$, $p<0.001$). Baseline serum surfactant protein D was not associated with either decline in FEV_1 ($r=0.004$, $p=0.866$), FEV_1% predicted ($r=0.004$, $p=0.837$) or FVC ($r=-0.033$, $p=0.117$). The median value of serum SP-D was similar between those individuals who had one or more exacerbations during 12 months of follow-up and those who had no exacerbations (121.5 vs 120.3 ng/mL, n=1093 and 795 respectively; p=0.062). Moreover there was no correlation between serum SP-D 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 ECLIPSE, there was a trend towards higher baseline median values of SP-D in those individuals that died during follow-up compared to those who remained alive (138.8 and 120.9 ng/mL, n=40 and 1848 respectively; p=0.125).

Serum SP-D 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 (C.I. 1.07, 1.39) for exacerbations for each 100 ng/ml increase in SP-D after adjusting for gender, FEV_1% predicted, reversibility and those individuals taking inhaled corticosteroids. This was more marked if the analysis was restricted to those individuals with a baseline SP-D in the upper quartile (OR 1.42, C.I. 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 values greater than the 99th percentile (382.7 ng/mL) were excluded (OR 1.58, C.I. 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 in the year prior to enrolment in the study. Serum SP-D remained associated with an increased risk of exacerbations (OR 1.23, C.I. 1.02, 1.49). The results were unchanged if the analysis was repeated with either diuretic or beta-blocker medication being included as confounding factors.

The 95th percentile of serum SP-D for the non-smokers was 175.5 ng/mL. This value was used to categorise the subjects with COPD as having either high or low levels of serum SP-D. The odds ratio (OR) for exacerbations of COPD was 1.30 (C.I. 1.03, 1.63) in individuals with high serum levels of SP-D after adjusting for gender, FEV_1% predicted and those individuals taking inhaled corticosteroids (neither age, smoking status, pack years smoked, nor reversibility were significant predictors in this model). Repeating this analysis with the 75th percentile of serum SP-D for COPD subjects (174.2 ng/mL) as a cut off to categorise the subjects with COPD gave similar results (OR 1.28,

CI 1.02, 1.61). Similar results were also obtained if exacerbations were defined based on the requirement for antibiotics (approximately 80% of all exacerbations; OR 1.31 C.I. 1.05, 1.64).

Assessment of the reproducibility of serum SP-D

It is important to know whether serum SP-D is a reproducible biomarker and so it was measured in an age-matched subgroup of 195 individuals with COPD, 36 smoker controls and 36 non-smoking controls selected from the ECLIPSE cohort (Table 2). Individuals with COPD and smoker controls were all former smokers to reduce the variability associated with smoking status. SP-D gave reproducible values in non-smokers, former smokers without airflow obstruction and across all severities of COPD when measured over a period of 3 months (Fig. 2 and Table 2; coefficient of repeatability 70.20 ng/mL, variability 26%).

Effect of prednisolone on serum SP-D in individuals with COPD

Serum levels of SP-D were similar in ECLIPSE regardless of background therapy including inhaled corticosteroids and long acting β_2-agonists. However, a modest fall in serum SP-D 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]. We tested the effect of administration of a systemic corticosteroid on serum SP-D in a study separate from ECLIPSE. We recruited 89 current or former-smokers diagnosed with chronic bronchitis and COPD and randomised them to receive either oral prednisolone or placebo. The groups were well matched for age, sex, lung function, the

degree of reversibility and pack-years smoked (Table 3). There were 5 withdrawals in the prednisolone group and 4 in the placebo group during the course of the study. Treatment with prednisolone resulted in a small increase relative to placebo in pre- and post- bronchodilator FEV_1 of 97 and 107 mL respectively (Fig. 3a); neither of these changes was statistically significant ($p=0.07$ and 0.06 respectively). However treatment with prednisolone resulted in a striking fall in serum SP-D from 126.0 to 82.1 ng/mL at 4 weeks ($p<0.001$; Fig. 3b). It remained low whilst the subjects were taking 20 mg/day 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 at baseline, 135.8 ng/mL at week 4) in the serum level of SP-D 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, IL-1β, Il-8, IL-6, MPO or MMP-9, data not shown).

Discussion

The ECLIPSE cohort was used to evaluate serum SP-D as a biomarker for COPD. The median serum level of SP-D was significantly higher in current and former smokers with COPD when compared to those without airflow obstruction. Serum SP-D was similar in men and women, it was not affected by the presence of chronic bronchitis and it did not correlate with either the radiologists' score of emphysema 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 levels of SP-D between individuals with COPD and smoker and non-smoker controls, whilst statistically significant, is not sufficiently large to use in a screening test to diagnose COPD.

The largest difference in serum levels of SP-D was between non-, current- and former smokers. Therefore serum SP-D is a powerful biomarker for smoking. Intrapulmonary levels of SP-D rise following the acute exposure of mice to cigarette smoke [23] but are lower in lung lavage 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, leak from the lung as a consequence of increased vascular permeability associated with inflammation. They are 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 can be derived from the non-smoking controls. It was striking that those individuals with COPD who had levels of SP-D that were greater than

the 95th percentile of normal controls had a greater risk of self-reported exacerbations. These symptoms were reported prospectively and are 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 concentrations of baseline serum SP-D with an even greater risk if the analysis was confined to those individuals in the upper quartile of baseline SP-D. It is possible that this effect is driven by a few outliers and so the analysis was repeated following the exclusion of individuals with the highest levels of serum SP-D. Once again serum SP-D was associated with exacerbations of COPD. Finally it is possible that serum SP-D is affected by co-morbidities and so the analysis was repeated with either diuretic or beta-blocker medication being included as confounding factors. This did not affect the results. Previous work has shown that plasma CRP [25] and serum amyloid A [26] are non-specific 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 levels of serum SP-D) are at greatest risk of exacerbations as 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 so serum SP-D may be useful at identifying those at greatest risk 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 was assessed in a different group of individuals in the ECLIPSE cohort. Levels showed 26% variability in non-smokers and former smokers with and without COPD over a 3 month interval. This variability may be higher in individuals with higher baseline levels of serum SP-D.

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 levels of SP-D whilst individuals with COPD received oral corticosteroids. Serum SP-D returned to baseline following the cessation of treatment. The change in serum levels of SP-D was 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, the expression of SP-D 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 in our cohort as most subjects were taking this medication.

The association of serum SP-D with COPD reported here is 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, systemic inflammation) during the 3 years 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 will be 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 levels of SP-D will provide an intermediate measure of disease modification in COPD.

In summary we have used a large cohort of individuals with COPD and smoking and non-smoking controls to show that median serum levels of SP-D are elevated, and predict exacerbations, in individuals with COPD. 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.

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Appendix 1. Principal investigators and centres participating in ECLIPSE (NCT00292552)

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Denmark: Jørgen Vestbo, Hvidovre.

Netherlands: Emiel Wouters, Horn-Maastricht.

New Zealand: Dean Quinn, Wellington.

Norway: Per Bakke, Bergen.

Slovenia: Mitja Kosnik, Golnik.

Spain: Alvar Agusti, Jaume Sauleda, Palma de Mallorca.

Ukraine: Yuri Feschenko, Kiev; Vladamir Gavrisyuk, Kiev; Lyudmila Yashina, Kiev; Nadezhda Monogarova, Donetsk.

United Kingdom: Peter Calverley, Liverpool; David Lomas, Cambridge; William MacNee, Edinburgh; David Singh, Manchester; Jadwiga Wedzicha, London.

United States of America: Antonio Anzueto, San Antonio, TX; Sidney Braman, Providence, RI; Richard Casaburi, Torrance CA; Bart Celli, Boston, MA; Glenn Giessel, Richmond, VA; Mark Gotfried, Phoenix, AZ; Gary Greenwald, Rancho Mirage, CA; Nicola Hanania, Houston, TX; Don Mahler, Lebanon, NH; Barry Make, Denver, CO; Stephen Rennard, Omaha, NE; Carolyn Rochester, New Haven, CT; Paul Scanlon, Rochester, MN; Dan Schuller, Omaha, NE; Frank Sciurba, Pittsburgh, PA; Amir

Sharafkhaneh, Houston, TX; Thomas Siler, St. Charles, MO, Edwin Silverman, Boston, MA; Adam Wanner, Miami, FL; Robert Wise, Baltimore, MD; Richard ZuWallack, Hartford, CT.

Steering Committee: Harvey Coxson (Canada), Lisa Edwards (GlaxoSmithKline, USA), Katharine Knobil (Co-chair, GlaxoSmithKline, UK), David Lomas (UK), William MacNee (UK), Edwin Silverman (USA), Ruth Tal-Singer (GlaxoSmithKline, USA), Jørgen Vestbo (Co-chair, Denmark), Julie Yates (GlaxoSmithKline, USA).

Scientific Committee: Alvar Agusti (Spain), Peter Calverley (UK), Bartolome Celli (USA), Courtney Crim (GlaxoSmithKline, USA), Gerry Hagan (GlaxoSmithKline, UK), William MacNee (Chair, UK), Stephen Rennard (USA), Ruth Tal-Singer (GlaxoSmithKline, USA), Emiel Wouters (The Netherlands), Julie Yates (GlaxoSmithKline, USA).

Appendix 2. Principal investigators and centres participating in the assessment of oral corticosteroids in individuals with COPD (NCT00379730)

Eric Bateman (Principal Investigator), Cape Town, South Africa; Pedro Elias, Mendoza, Argentina; Fabian Galleguillos, Santiago, Chile; Dean Quinn, Wellington, New Zealand; Liliana Vicherat, Santiago, Chile.

Figure legends

Fig. 1a. Measurement of serum SP-D in individuals with COPD and controls. The number of individuals in each group is shown in brackets. The bars are median and interquartile range, the solid dot is the mean. The number of current smokers in each group was: smoking controls 201, all COPD individuals 746, COPD GOLD stage II 334, GOLD stage III 324 and GOLD stage IV 76. Fig. 1b. Effect of current smoking on SP-D levels in smoking controls and individuals with COPD. Individuals with COPD are shown in total and divided into groups based on the GOLD classification. The number of current smokers in each group is the same as in Fig. 1a.

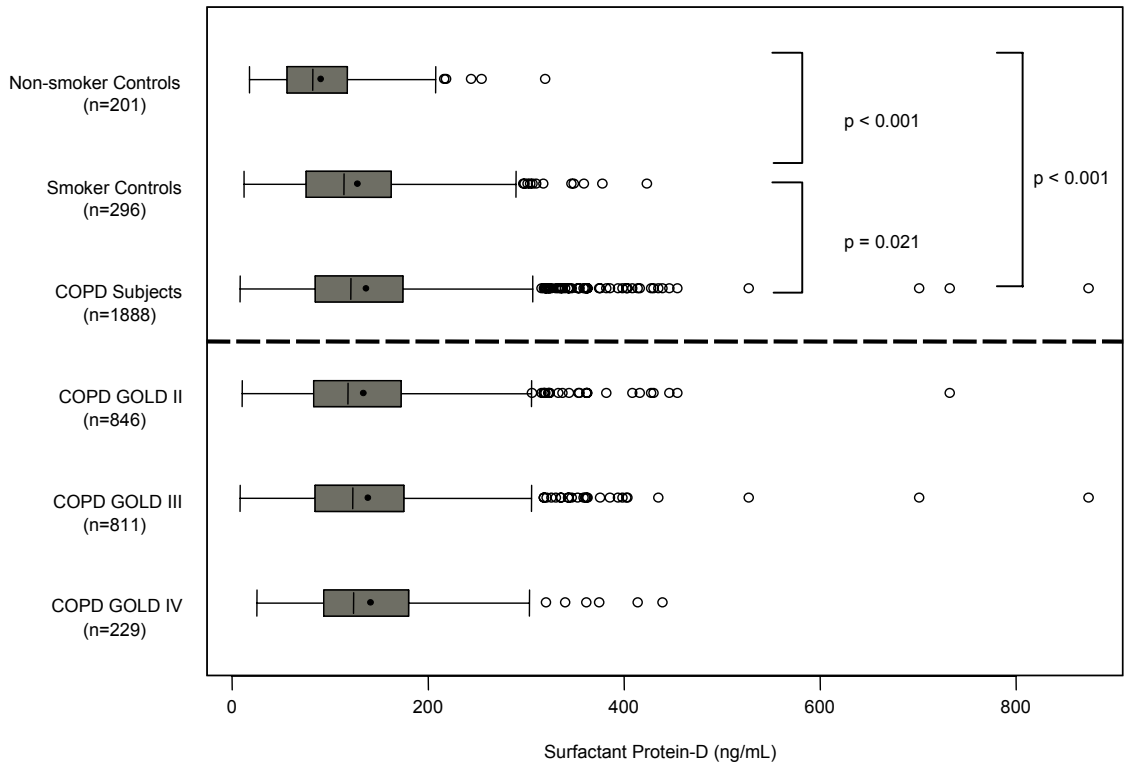

Non-smoker Controls
(n=201)
Smoker Controls
(n=296)
COPD Subjects
(n=1888)
COPD GOLD II
(n=846)
COPD GOLD III
(n=811)
COPD GOLD IV
(n=229)
p < 0.001
p = 0.021
p < 0.001
0
200
400
600
800
Surfactant Protein-D (ng/mL)

Figure 1a

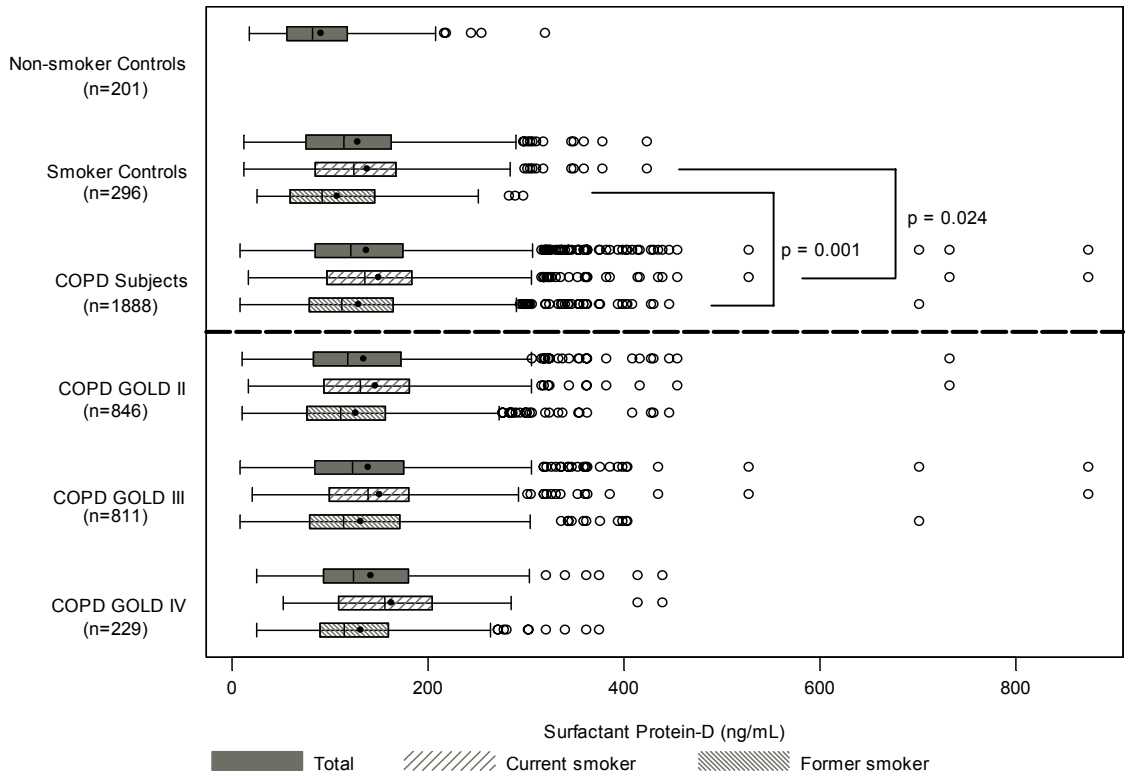


Figure 1b

Fig. 2. Bland-Altman plot to assess the reproducibility of serum levels of SP-D at baseline and 3 months in 267 individuals with and without COPD. The broken lines represent 95% limits of agreement. Bias is -2.23 and coefficient of repeatability is 70.20 ng/mL.

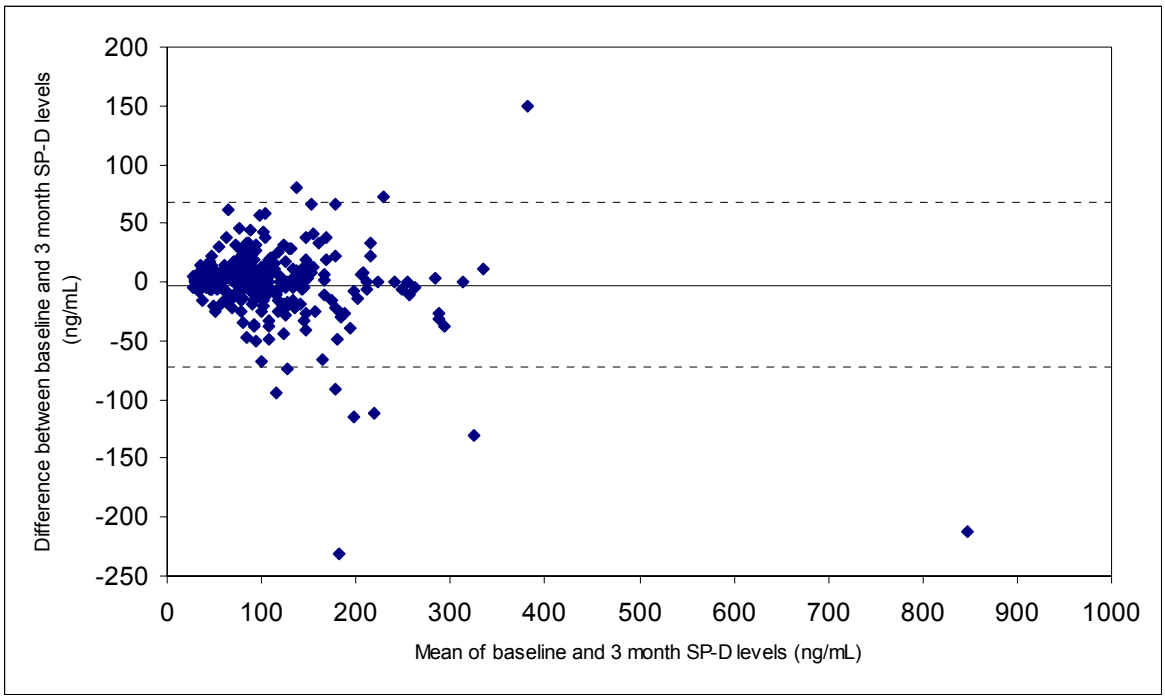
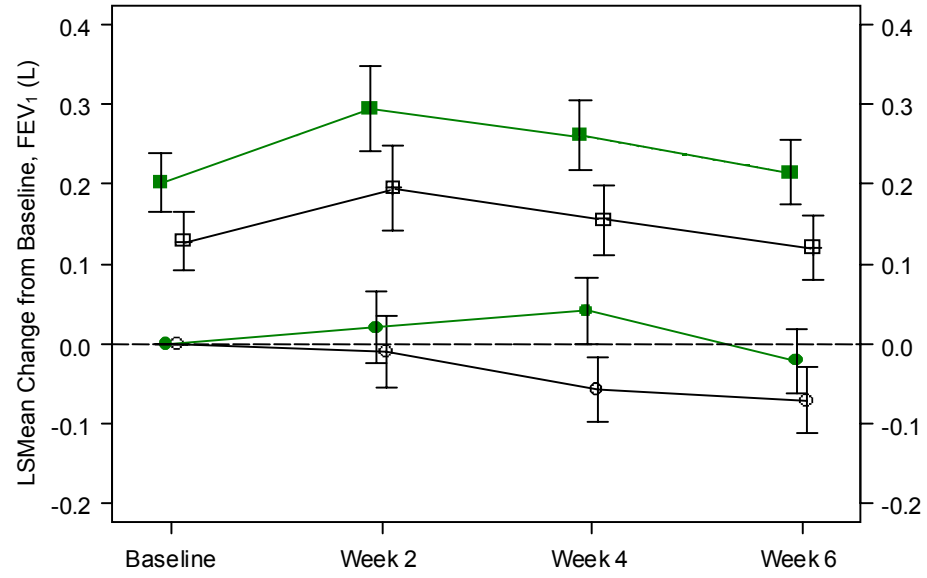


Figure 2

Fig. 3. Effect of oral corticosteroids on FEV_1 (Fig. 3a) and serum SP-D (Fig. 3b) in individuals with COPD. The least squares means (LSMeans) were adjusted for baseline and study site in the analysis model. Individuals with COPD were randomised to prednisolone (green line) or placebo (black line). The prednisolone group received 20 mg/day prednisolone for 4 weeks, 10 mg/day prednisolone for 1 week and 5 mg/day prednisolone for 1 week. The effect of prednisolone on FEV_1 is shown before (circles)

and after (squares) the administration of 180 mcg of salbutamol. The numbers of individuals (N) at each time point is shown in black (placebo) and green (prednisolone) in Fig. 3b. The data were analysed by intention to treat and are shown as mean and standard error, **p<0.001.



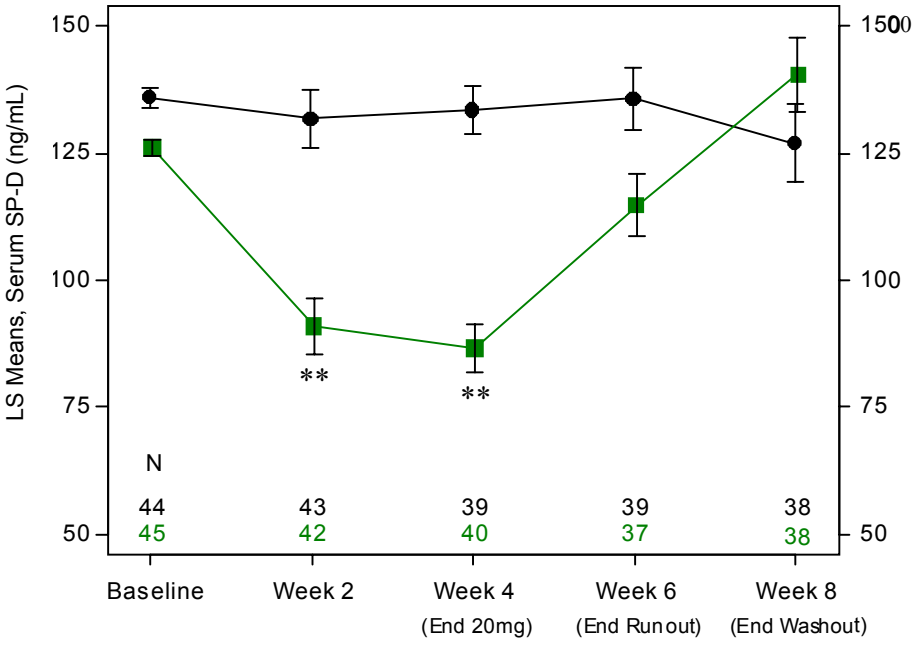


Figure 3a (top) and 3b (bottom)

	COPD subjects	Smoker controls	Non-smoker controls	p value†
Number	1888	296	201	
Age	63.4 (7.2)	54.7 (8.9)	53.2 (8.6)	<0.001
Male (%)	1222 (65)	161 (54)	74 (37)	0.001
Smoking history, pack-years	49.2 (27.3)	32.0 (22.1)	0.4 (0.5)	<0.001
Current smoker (%)	746 (40)	201 (68)	0	<0.001
FEV_1 (L)	1.4 (0.5)	3.4 (0.8)	3.3 (0.8)	<0.001
FEV_1 % pred	48.7 (15.5)	108.6 (12.1)	114.8 (14.0)	<0.001
FEV_1/FVC	0.45 (0.11)	0.79 (0.05)	0.81 (0.05)	<0.001
CT scans (n)	1496	260	165	
% Low attenuation area (< -950 HU)	16.9 (11.8)	2.2 (2.9)	3.9 (4.0)	<0.001
SP-D ng/mL, median (IQR)	121.1 (89.4)	114.3 (86.8)	82.2 (61.6)	0.021

Table 1. The assessment of serum SP-D in individuals with and without COPD. The lung function measurements are following the administration of 180 mcg of salbutamol. CT scans is the number of CT scans available for qualitative analysis to assess the percentage of the lungs with a density of -950 HU. All values are numbers or mean and standard deviation (in brackets) unless otherwise stated. †p values for difference between

COPD subjects and smoker controls. All the parameters measured were significantly different between individuals with COPD and non-smoker controls ($p<0.001$).

	COPD subjects	Smoker controls	Non-smoker controls	p value†
Number	195	36	36	
Age	64.5 (6.0)	60.8 (7.7)	59.7 (8.8)	0.002
Male (%)	141 (72)	24 (67)	14 (39)	0.492
Smoking history, pack-years	45.8 (27.2)	29.8 (16.5)	1 (0)	0.001
FEV_1 (L)	1.2 (0.5)	3.2 (0.6)	3.1 (0.7)	<0.001
FEV_1 % pred	43.9 (16.9)	108.9 (11.8)	115.8 (12.0)	<0.001
FEV_1/FVC	0.40 (0.12)	0.80 (0.06)	0.80 (0.05)	<0.001
CT scans (n)	178	29	32	
% Low attenuation area (<-950 HU)	22.6 (13.5)	4.5 (4.4)	5.4 (5.5)	<0.001
Baseline SP-D ng/mL, median (IQR)	103.6 (66.3)	96.5 (52.8)	71.8 (50.7)	0.116
Number of SP-D results at 3 months	181	35	34	
3 month SP-D ng/mL, median (IQR)	105.1 (74.7)	95.5 (60.7)	77.5 (47.1)	0.232

Table 2. Assessment of the reproducibility of serum SP-D. The lung function measurements are following the administration of 180 mcg of salbutamol. All the smoking controls and individuals with COPD were former smokers. CT scans is the

number of CT scans available for qualitative analysis to assess the percentage of the lungs with a density of -950 HU. All values are number or mean and standard deviation (in brackets) unless otherwise stated. †p values for difference between COPD subjects and smoker controls. All the parameters measured were significantly different between individuals with COPD and non-smoker controls ($p<0.001$).

	Placebo	Prednisolone
Number	44	45
Age, yrs	62.8 (8.4)	62.6 (9.1)
Male (%)	32 (73)	35 (78)
FEV1 (L)	1.35 (0.56)	1.33 (0.56)
FEV1 %Predicted	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)
Pack-years smoked	53.4 (35.1)	48.4 (24.5)
Current smoker (%)	21 (48)	19 (42)
Number taking salbutamol (%)	35 (80)	36 (80)
Number taking ipratropium bromide (%)	17 (39)	25 (56)
Number with CT scan	36	40
Emphysema on CT scan (%)	34 (94)	36 (92)

Table 3. Demographics and baseline characteristics of individuals with COPD randomised to receive either oral corticosteroids or placebo. Reversibility was defined as an increase in FEV_1 following 180 mcg of salbutamol. The number of individuals with any emphysema on their CT scan was determined from the radiologists score. There were 5 withdrawals in the prednisolone group and 4 in the placebo group. All values are number or mean and standard deviation (in brackets) unless otherwise stated.