Increased serum SP-D in identification of high-risk smokers at high risk of COPD

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Author contributions:
CD, KOK, JV and GLS conceived and designed the analyses
ILT collected the lung function data
CD and FW performed the analyses and wrote first draft
All revised and approved before submission
Abstract

Pulmonary surfactant protein D (SP-D) is an important component of the pulmonary innate immune system with the ability to dampen cigarette smoke-induced lung inflammation. However, cigarette smoking mediates translocation of SP-D from the lung to the blood, and serum SP-D (sSP-D) has therefore previously been suggested as marker for smoke-induced lung injury. In support of this notion, associations between high sSP-D and low lung function measurements have previously been demonstrated in smokers and in COPD. The present investigations employ a 12-year longitudinal Danish twin study to test the hypothesis that baseline sSP-D variation has the capacity to identify smokers with normal baseline lung function who are in high risk of significant future smoke-induced lung function decline. We find that sSP-D is significantly increased in those with normal lung function at baseline that develop lung function decline during follow up compared to those who stay lung healthy. Moreover, we demonstrate that it is the smoke-induced baseline sSP-D level, and not the constitutional level, which has capacity as biomarker, and which is linearly increased with the decline in lung function during follow up. In conclusion, we here present first observation of increased sSP-D for identification of high-risk smokers.

Keywords

Surfactant protein D, tobacco smoking, chronic obstructive lung disease, twin study, biomarker
Pulmonary surfactant protein D (SP-D) is a soluble innate immune-factor synthesized by airway epithelium (1) and is recognized to be protective against smoke-induced airway inflammation in vivo (2). However, SP-D is translocated from bronchoalveolar fluid into serum with lung injury or tobacco smoking resulting in lowered pulmonary levels and increased serum levels (3-7). This translocation is expected to result from inflammation-induced alveolo-capillary leakage and from partial decomposition of the multimeric SP-D into more easily diffusible low molecular weight forms (8). Serum SP-D (sSP-D) has been tested as biomarker for various inflammatory lung diseases including chronic obstructive lung disease (COPD) (9).

US Food and Drug Administration has only approved fibrinogen as biomarker for COPD prognosis (FDA.gov). However, a broader search is ongoing because efficacious biomarkers are expected to help risk stratification, may allow clinical trials to be catered to the patients most likely to progress, and may provide a more accurate prognosis. Newly suggested biomarkers include diverse types of factors such as anti-inflammatory mediators (10), microvesicles (11, 12), and micro-RNA (13). Data from the large, longitudinal, multicenter studies ECLIPSE and COPDGene have used identical assay platforms to support that the inclusion of circulatory SP-D into biomarker panels improved the predictive value of lung function decline, progression of emphysema and mortality in COPD (14). However, no combination of tested biomarkers added significant value to predicting an individual’s future risk of COPD exacerbations over clinical variables and the biomarker variations only explained a limited fraction of variation in additional endpoints (14, 15). One major limitation for the use of circulatory SP-D as biomarker in COPD is that tobacco smoking in some studies appears to induce circulatory SP-D to a similar degree as
COPD per se (16-19). As a result, the prognostic efficacy may be difficult to sort out in cohorts of smoking COPD patients. However, the clear induction of circulatory SP-D with tobacco smoking has previously forged the question if sSP-D could reflect subclinical smoke induced lung damage. We previously reported on the cross-sectional association between sSP-D and the forced expiratory volume in 1 second (FEV₁) as well as forced vital capacity (FVC) and observed a significant relationship in smokers only (20). In the present study, the primary objective was to examine if the baseline sSP-D variation has the capacity to identify those smokers with normal baseline lung function who will experience a clinically relevant smoke-induced lung function decline over 12-years of follow-up.
Materials and methods

Participants were originally recruited from the nationwide, population-based, longitudinal Danish Twin Registry during 1997–2000 to examine the genetic and environmental backgrounds in the development of insulin resistance, abdominal obesity and cardiovascular risk factors; i.e., the GEMINAKAR I (GK-I) study as described previously (21-24). Twins who consented to participation were followed up in GEMINAKAR II (GK-II) during 2010 to 2012 as described by Verhulst-S et al., 2016 (25). At enrollment, exclusion criteria included known diabetes or cardiovascular disease, conditions making a progressive maximal bicycle test impossible, pregnancy, and breast feeding. The cohort consisted of 756 complete twin pairs (783 females, 729 males, among them, 311 monozygotic (MZ) and 445 dizygotic (DZ) twin pairs) who underwent an extensive full day clinical examination of a variety of phenotypes at baseline. Furthermore, at baseline co-twins had the clinical examination at the same day, while at follow-up participants were visited at their home and mostly not at the same day as their co-twin. In this study, we included 544 subjects with available lung function test data at both baseline and follow up, information on SP-D concentration at baseline as well as complete information on smoking and covariates. Among these 544 individuals, there were 187 complete twin pairs (374 individuals) and 170 singletons. The GEMINAKAR studies were approved by the local scientific committee of the Region of Southern Denmark (baseline, S-VF-19970271; follow-up, S-20090065) and by the Danish Data protection Board (baseline, 1999-1200-441; follow-up, 2009-41-2990). Written informed consent was obtained from all participants in the study. The present study was approved by SDU Research & Innovation Organization in compliance with the General Data Protection Regulation (j.no. 11.087).
Lung function testing, blood sample collection, and sSP-D analysis

Spirometry was conducted on both twins in a pair on the same day at two different investigational centers at baseline, according to the most recent guidelines at that time \( (26) \). At baseline, spirometry was performed using a Vitalograph dry-bellow spirometer in Copenhagen and a noncommercial pneumotachograph in Odense. Prior or present medical history of respiratory disease and information regarding tobacco smoking status (never, previous, or current smoker) were obtained by questionnaire. At follow-up, each participant was visited at home in a mobile examination center where forced spirometry was conducted using Microlab Spirometer. Certified study nurses performed the spirometry measures at both time points. The participant’s height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. BMI was calculated as bodyweight in kg divided by squared height in m.

Blood was collected after an overnight fast and serum stored at \(-80^\circ C\) until analysis. sSP-D was measured using a sandwich ELISA, as previously described \( (6, 27) \). All analyses were performed in duplicate. The maximal accepted CV\% was 10 %.

Statistical analysis

Continuous data are presented as mean (SD) or median (95% CI) according to distribution. Categorical data are presented as n (%). ANOVA or Kruskall-Wallis test was used for comparisons of continuous data whatever appropriate. Distribution of sSP-D concentration was skewed to the right and was therefore log-10 transformed when included in the regression models in order not to violate model assumptions.
The participants were categorized into three groups according to their FEV$_1$/FVC ratio at both baseline and follow-up using GOLD criteria (28); group 1, FEV$_1$/FVC $\geq$70% at baseline and follow-up; group 2, FEV$_1$/FVC $\geq$70% at baseline and FEV$_1$/FVC <70% at follow-up; and group 3, FEV$_1$/FVC <70% at baseline and follow-up. Thus, group 1 comprises subjects with normal lung function both at baseline and follow up. Group 2 were subjects with normal lung function at baseline and airflow limitation at follow up. Group 3 were subjects with airflow limitation at both baseline and follow up.

The primary outcome was annual change in FEV$_1$ during follow-up, and the secondary outcome was incidence of airflow limitation at follow-up. We used multivariable linear regression models to examine the main association of sSP-D concentration and changes in FEV$_1$ and tested the interaction between sSP-D and smoking by including the interaction term sSP-D $\times$ smoking in the model. Models were adjusted for baseline age, height, weight, sex, and FEV$_1$. Estimates from the regression models were back transformed to express percent difference in annual FEV$_1$ decline with a doubling in sSP-D concentrations. We used multivariable logistic regression to examine the association between sSP-D and odds of airflow limitation (FEV$_1$/FVC <70%) at follow-up to calculate the OR and 95% CI. Subjects with diagnosed COPD at baseline were excluded from analyses.

Model assumptions about homoscedasticity and normal distribution of the residuals were inspected visually using plots of residuals against fitted values and quantile-normal plots of the standardized residuals, respectively.

All analyses were performed using STATA/IC version 16.1, Stata Corp, Texas, USA, and a 5% level of significance were used when testing associations and interactions.
Results

Lung function measurements, numbers of smokers and sSP-D levels at baseline and follow up

Based on the available lung functions measures and estimated COPD status, the final study sample included 538 participants, as we further excluded six subjects with FEV₁/FVC < 70% at baseline but ≥ 70% at follow-up. Thus, the present study included 36 (6.1%) participants with airflow limitation at follow-up (group 2) and 69 (12.8%) at baseline and follow up (group 2+3).

Tables 1 and 2 display the general characteristics of the participants in the GEMINAKAR cohort at baseline and follow-up according to lung function during study.

Age was significantly lower in group 1 compared to group 3, whereas there was no significant difference between groups 1 and 2. Height, weight and BMI did not differ between groups. The numbers of smokers were significantly lower in group 1 (approx. 25%) compared to groups 2 and 3 both at baseline and at follow up (> 50%) but did not differ between groups 2 and 3.

sSP-D was measured at baseline and was significantly higher in both group 2 (1.3-fold) and group 3 (1.5-fold) relative to group 1. sSP-D did not differ significantly between groups 2 and 3 but was insignificantly lower in group 2 relative to group 3.

As expected, groups 2 and 3 had significantly reduced FEV₁ % of predicted compared with group 1 at baseline and follow up. Further, group 3 had significantly lower FEV₁ % of predicted compared to group 1 at baseline.

High baseline sSP-D is associated with future lung function decline in smokers

sSP-D was significantly higher in smokers (1,288.6 ng/mL (1,113.5 – 1,452.7 ng/mL)) (median (95% CI)) compared to non-smokers (833.2 ng/mL (792.0 – 898.7 ng/ml)) and previous smokers (877.3 ng/mL (806.1 – 1,004.4) ng/mL)) (both p<0.0001). Also, subjects without airflow
limitation at any time (group 1) had significantly lower sSP-D concentration at baseline compared with both group 2 and 3 (Table 1).

Among participants without airflow limitation at baseline, FEV$_1$ decreased on average -28.7 mL/year (95% CI -32.2 – -26.5 mL/year), however the decline among smokers was larger than among never-smokers (-35.3 mL/year (-42.1 – -32.2 mL/year) vs -25.4 mL/year (-27.4 – -22.8 mL/year), (p<0.001)) (Table 2). We found a significant interaction between smoking status and sSP-D on FEV$_1$ decline (p-interaction=0.002). Thus, SP-D was significantly associated with lung function decline during follow up but only in smokers (Table 3, Figure 1), where a doubling in sSP-D concentration was associated with a 9.5 % (95% CI, 3.8 – 15.3%) larger decline in FEV$_1$.

**High baseline serum SP-D is associated to future COPD in smokers**

For the investigation of whether SP-D is associated with incident COPD over a 12-year period, we excluded participants with airflow limitation at baseline (N=33, group 3) and the population then consisted of 505 twins. During follow-up, 36 (7.1%) incident COPD cases were documented by the spirometric criterion for air flow limitation of whom 18 (50%) were smokers at baseline.

After adjusting for potential confounders, the analyses showed that higher baseline sSP-D concentration was associated with an increased COPD risk during follow-up, but mostly in smokers (Table 4). However, the interaction-term between SP-D and smoking was not significant (p-interaction=0.195) which could be due to the reduced statistical power caused by the small numbers.
Discussion

In the present longitudinal findings from the GEMINAKAR twin cohort among subjects without airflow limitation at baseline, higher sSP-D concentration was associated with a larger annual decline in FEV1 in smokers compared with non-smokers. Furthermore, higher sSP-D concentration at baseline was associated with increased COPD risk, primarily in smokers although not statistically significant.

The data show that it is the smoke-induced SP-D levels, and not the constitutive levels, which have capacity as biomarker, and which is linearly increased with the decline in lung function during a period of 12 years. This finding was independent of sex, age, height and weight, which are factors recognized to affect sSP-D (6) and support the biomarker efficacy in risk assessment for subclinical tobacco smoke-induced lung damage and identification of high-risk smokers for intensified smoke-cessation efforts and early pulmonary treatment intervention. The observed relations suggest that the relative risk for smokers of developing COPD in a 12-year period was increased by 9.5% per doubling of sSP-D.

It was recently demonstrated that persons without obstruction who have a baseline value for FEV1 at the low end of the normal range, a reduction in FEV1 that exceeds 40 ml per year over an 18-month period is associated with an increase by a factor of 36 in the 5-year risk of COPD (29). In comparison, smoke-induced sSP-D appears as a weaker prognostic marker. However, the strength of sSP-D is that measurement of high levels may immediately identify those with “pre-COPD” with the aim to encourage adopting a healthy lifestyle, avoid injurious exposures, and to be monitored with frequent spirometry. It is noteworthy that almost half of the subjects with airflow limitation at follow-up already had spirometric obstruction at baseline. This emphasizes
the need to view COPD as a disease that often has its roots early in life and that many patients
have COPD that has developed through a trajectory with early loss of lung function and likely
only a modest decline in FEV\textsubscript{1} (30). It is possible that this could be more frequent in twins than
in singletons as a result of earlier birth and lower birthweight.

sSP-D has been examined in other cohorts without finding an association between the biomarker
and subsequent decline in FEV\textsubscript{1} (14, 31). However, most of the subjects in these cohorts had
established COPD, underlining that sSP-D is a biomarker of early changes. The early
identification of high-risk smokers may furthermore be important due to the close relation
between smoking or COPD and increased hazard ratio for cardiovascular disease mortality in
COPD (32, 33). Increased systemic sSP-D is in itself independently associated to CVD outcomes
(33-36) and sSP-D-deficiency is demonstrated to be surprisingly protective in development of
atherosclerosis in vivo (37-39).

Limitations of the analysis include that the finding warrants replication in an independent
population-based cohort. The strengths of our study include the large sample size, the long-
follow-up period and use of standardized lung function measurements by certified nurses.
However, the study is of observational nature and the findings may thus be the result of reverse
confounding although this is unlikely as participants were without airflow limitation at baseline.
We cannot exclude residual confounding. However, we have adjusted for known potential
factors associated with risk of airflow limitation and sSP-D. According to GOLD (28), the
diagnosis of clinical COPD requires both airflow limitation and symptoms in an individual with
relevant exposures. In this study we equated airflow limitation to COPD; although this may
slightly overestimate incidence it has been done in other cohort studies as well (30, 40).
In conclusion, we here present a first observation of serum SP-D as a marker in smokers for increased risk of developing COPD.
Acknowledgement

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References


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D. D. Sin, Surfactant Protein D Deficiency Suppresses Systemic Inflammation and Reduces
reactive protein and chronic obstructive pulmonary disease: a Mendelian randomisation
Figure Legend

Figure 1. Associations between serum surfactant protein (SP) D with yearly decline of FEV1 (A) and FVC (B) by smoking status among participants in GEMINAKAR cohort.
Predicted FEV₁ decline (mL/year)

-20  -15  -10  -5  0  5  10  15  20  25

Predicted FVC decline (mL/year)

-50  -40  -30  -20  -10  0  10  20  30  40

log-transformed sSP-D (per 100 ng/mL)

- EX-smokers vs never smokers
- Smokers vs never smokers
Table 1 Baseline characteristics of the participants in GEMINAKAR by lung function during study

<table>
<thead>
<tr>
<th></th>
<th>Group 1a</th>
<th>Group 2a</th>
<th>Group 3a</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=469</td>
<td>n=36</td>
<td>n=33</td>
<td></td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>251 (53.5%)</td>
<td>15 (61.3%)</td>
<td>21 (60.0%)</td>
<td>0.071</td>
</tr>
<tr>
<td>Age, years</td>
<td>37 (36 - 39)</td>
<td>39.5 (36.0 – 48.7)</td>
<td>44 (40.3 – 48.0)</td>
<td>0.006§</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 (171 – 173)</td>
<td>175 (170 – 178)</td>
<td>173 (171 – 178)</td>
<td>0.1124</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.3 (70.6 – 73.7)</td>
<td>71.4 (64.6 – 77.0)</td>
<td>72.9 (65.7 – 78.8)</td>
<td>0.9452</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.5 (3.3)</td>
<td>23.8 (2.8)</td>
<td>23.4 (3.8)</td>
<td>0.1378</td>
</tr>
<tr>
<td>FEV₁ Med, L</td>
<td>3.5 (3.4-3.6)</td>
<td>3.4 (3.1-3.8)</td>
<td>2.9 (2.5 - 3.1)</td>
<td>&lt;0.0001d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.010c</td>
</tr>
<tr>
<td>% of predicted</td>
<td>97.7 (96.2-99.2)</td>
<td>89.3 (86.7-95.8)</td>
<td>77.2 (76.1-84.2)</td>
<td>&lt;0.001d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001e</td>
</tr>
<tr>
<td>FVC Med, L</td>
<td>4.4 (4.3-4.5)</td>
<td>4.7 (4.1 – 5.1)</td>
<td>4.5 (3.8 – 4.9)</td>
<td>0.610</td>
</tr>
<tr>
<td>% of predicted</td>
<td>99.2 (98.4-100.8)</td>
<td>97.0 (93.5-103.6)</td>
<td>96.1 (91.4-104.5)</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001c</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>79.7 (79.2-80.1)</td>
<td>73.9 (73.2-76.0)</td>
<td>65.6 (63.8-67.4)</td>
<td>&lt;0.0001d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001c</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>118 (25.2%)</td>
<td>18 (50.0%)</td>
<td>20 (60.6%)</td>
<td></td>
</tr>
<tr>
<td>sSP-D, ng/ml</td>
<td>894.4 (845.6-944.0)</td>
<td>1,134.8 (851.4 – 1,506.3)</td>
<td>1,413.5 (1,092.7 – 1,783.0)</td>
<td>0.0049c</td>
</tr>
</tbody>
</table>

Continuous data are presented as median (95%CI). Categorical data are presented as n (%).

aGroup 1, FEV₁/FVC ≥70% at GKI and GKII; Group 2, FEV₁/FVC ≥70% at GKI and FEV₁/FVC <70% at GKII; Group 3, FEV₁/FVC <70% at GKI and GKII.

bP values regard comparisons between Group 1 and Group 2c; Group 1 and Group 3d; and Group 2 and Group 3e, respectively.

BMI = Body mass index.
FEV₁ = Forced expiration in one second.
FVC = Forced vital capacity.
sSP-D = Serum surfactant protein D.
### Table 2: GKII follow up characteristics of the participants in GEMINAKAR by lung function during study

<table>
<thead>
<tr>
<th></th>
<th>Group 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group 3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=469</td>
<td>n=36</td>
<td>n=33</td>
<td></td>
</tr>
<tr>
<td>Follow-up time, years</td>
<td>11.9 (11.7 – 12.0)</td>
<td>11.7 (11.4 – 12.0)</td>
<td>11.8 (11.4 – 12.2)</td>
<td>0.816</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.9 (73.2 – 76.7)</td>
<td>72.0 (69.3 – 84.4)</td>
<td>75.7 (67.8 – 82.0)</td>
<td>0.971</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>25.4 (25.0–25.6)</td>
<td>23.6 (21.6–26.0)</td>
<td>24.6 (24.0–26.2)</td>
<td>0.260</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median, L</td>
<td>3.2 (3.1–3.3)</td>
<td>2.8 (2.3 –3.3)</td>
<td>2.4 (1.9 – 2.9)</td>
</tr>
<tr>
<td></td>
<td>% of predicted</td>
<td>100.1 (99.2–101.8)</td>
<td>83.1 (77.8–90.9)</td>
<td>77.6 (65.5 – 88.1)</td>
</tr>
<tr>
<td></td>
<td>ΔFEV&lt;sub&gt;1&lt;/sub&gt;, mL/year</td>
<td>-27.1 (-30.4 – -25.0)</td>
<td>-38.1 (-55.5 – -25.7)</td>
<td>-42.9 (-59.3 – -17.5)</td>
</tr>
<tr>
<td>FVC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median, L</td>
<td>4.1 (3.9–4.2)</td>
<td>4.2 (3.5–4.7)</td>
<td>3.9 (3.2–4.5)</td>
</tr>
<tr>
<td></td>
<td>% of predicted</td>
<td>99.1 (97.8–100.4)</td>
<td>97.6 (85.5–105.0)</td>
<td>93.4 (87.9–102.9)</td>
</tr>
<tr>
<td></td>
<td>ΔFVC, mL/year</td>
<td>-26.0 (-30.0 – -23.7)</td>
<td>-38.1 (-55.5 – -25.7)</td>
<td>34.7 (-67.0 – -21.4)</td>
</tr>
<tr>
<td></td>
<td>ΔFVC, mL/year</td>
<td></td>
<td></td>
<td>0.005&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC, %</td>
<td>77.8 (77.2–78.4)</td>
<td>67.3 (64.6–68.3)</td>
<td>60.2 (58.0–65.7)</td>
</tr>
<tr>
<td></td>
<td>Delta FEV&lt;sub&gt;1&lt;/sub&gt;/FVC, %</td>
<td>-1.9 (-2.4– -1.6)</td>
<td>-8.5 (-10.3– -7.5)</td>
<td>-2.8 (-6.6 – -0.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Continuous data are presented as median (95%CI). Categorical data are presented as n (%).

<sup>a</sup>Group 1, FEV<sub>1</sub>/FVC ≥70% at GKI and GKII; Group 2, FEV<sub>1</sub>/FVC ≥70% at GKI and FEV<sub>1</sub>/FVC <70% at GKII; Group 3, FEV<sub>1</sub>/FVC <70% at GKI and GKII.

<sup>b</sup>P values regard comparisons between Group 1 and Group 2<sup>c</sup>; Group 1 and Group 3<sup>d</sup>; and Group 2 and Group 3<sup>e</sup>, respectively

BMI = Body mass index.

FEV<sub>1</sub> = Forced expiration in one second.

FVC = Forced vital capacity.

sSP-D = Serum surfactant protein D.
Table 3 Percent difference in lung function decline (FEV₁) per year with a doubling of SPD concentration at baseline during 12 years of follow-up in 505 subjects without COPD according to smoking status

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>% difference</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among non-smokers</td>
<td>274</td>
<td>-1.8</td>
<td>-5.4 – 1.8</td>
<td>0.318</td>
</tr>
<tr>
<td>Among previous smokers</td>
<td>95</td>
<td>-3.2</td>
<td>-10.1 – 3.8</td>
<td>0.367</td>
</tr>
<tr>
<td>Among smokers</td>
<td>136</td>
<td>-9.5</td>
<td>-15.3 – -3.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Multivariable models were adjusted for age, height, weight, sex, and FEV₁ at baseline.
Table 4 Odds ratio of COPD with a doubling in SP-D concentration by smoking during 12 years of follow-up in 505 subjects without COPD

<table>
<thead>
<tr>
<th></th>
<th>N cases/ N total</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among non-smokers</td>
<td>11/274</td>
<td>1.02</td>
<td>0.76 – 1.38</td>
<td>0.880</td>
</tr>
<tr>
<td>Among previous smokers</td>
<td>7/95</td>
<td>1.13</td>
<td>0.70 – 1.82</td>
<td>0.628</td>
</tr>
<tr>
<td>Among smokers</td>
<td>18/136</td>
<td>1.42</td>
<td>0.96 – 2.11</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Multivariable models were adjusted for age, height, weight, sex, and FEV₁ at baseline