Plasma pro-surfactant protein B and lung function decline in smokers

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ABSTRACT Plasma pro-surfactant protein B (pro-SFTPB) levels have recently been shown to predict the development of lung cancer in current and ex-smokers, but the ability of pro-SFTPB to predict measures of chronic obstructive pulmonary disease (COPD) severity is unknown. We evaluated the performance characteristics of pro-SFTPB as a biomarker of lung function decline in a population of current and ex-smokers.

Plasma pro-SFTPB levels were measured in 2503 current and ex-smokers enrolled in the Pan-Canadian Early Detection of Lung Cancer Study. Linear regression was performed to determine the relationship of pro-SFTPB levels to changes in forced expiratory volume in 1 s (FEV_1) over a 2-year period as well as to baseline FEV_1 and the burden of emphysema observed in computed tomography (CT) scans.

Plasma pro-SFTPB levels were inversely related to both FEV_1 % predicted ($p=0.024$) and FEV_1/forced vital capacity (FVC) ($p<0.001$), and were positively related to the burden of emphysema on CT scans ($p<0.001$). Higher plasma pro-SFTPB levels were also associated with a more rapid decline in FEV_1 at 1 year ($p=0.024$) and over 2 years of follow-up ($p=0.004$).

Higher plasma pro-SFTPB levels are associated with increased severity of airflow limitation and accelerated decline in lung function. Pro-SFTPB is a promising biomarker for COPD severity and progression.

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Introduction

Chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of morbidity and mortality in the USA [1], yet efforts to treat the disease effectively remain elusive, in part because of a scarcity of disease-modifying treatments and our inability to risk-stratify patients accurately for disease progression. The US Food and Drug Administration currently endorses the use of serial spirometric measures such as forced expiratory volume in 1 s (FEV_1) as a primary efficacy end-point for disease-modifying drugs treating COPD. FEV_1 measurements, while useful for diagnosis, are nonetheless highly variable. For clinical trials evaluating COPD therapies, the use of FEV_1 measurements requires the enrolment of thousands of patients over a long period of time to effectively assess disease progression. A simple and robust blood biomarker of lung function decline would be paradigm shifting, for both patient care as well as drug development. The search for biomarkers to reflect disease progression accurately in COPD, however, has yielded mixed results, with a number of systemic inflammatory markers, such as C-reactive protein and fibrinogen, lacking the desired specificity for useful clinical application [2, 3]. Instead, lung-specific proteins, such as surfactant protein D [4] and club cell protein 16 [5], may carry greater promise in their ability to better reflect the disease environment in question.

Surfactant protein B (SFTPB) is an 8-kD protein that is synthesised exclusively by type 2 alveolar pneumocytes and nonciliated bronchiolar cells. Its predominant role in the lung is to decrease the surface tension at the air–liquid interface. Specifically, SFTPB achieves this by promoting the adsorption and spread of phospholipids [6] and by preventing plasma proteins from disrupting these phospholipids [7]. In addition, its anti-inflammatory [8] and anti-oxidant properties [9] have made it a useful protein to study within the context of lung diseases such as acute respiratory distress syndrome [10], neonatal respiratory distress [11] and lung cancer [12]. Although its hydrophobic properties make it exceedingly difficult to measure reliably in plasma or bronchoalveolar lavage fluid, its precursor, pro-surfactant protein B (pro-SFTPB), is a hydrophilic 42-kD protein that is cleaved at the N and C terminus into the mature SFTPB form and secreted into the alveolar space. Recent detection of the N-terminal pro-peptide of SFTPB by mass spectrometry has allowed the development of a sandwich ELISA for pro-SFTPB that has subsequently been validated for prediction of the development of lung cancer through measurement in plasma samples [13]. Whether pro-SFTPB can demonstrate similar usefulness in COPD remains unknown. In this study, we investigated whether plasma levels of pro-SFTPB are related to lung function and to declines in FEV_1 over time.

Methods

Study population

The patient population for this study was derived from the multicentre Pan-Canadian Early Detection of Lung Cancer (Pan-Can) Study (NCT00751660; Screening Methods in Finding Lung Cancer Early in Current or Former Smokers). Details of this cohort have been published previously [14]. Patients enrolled in this study had to meet the following criteria: age 50–75 years, current or former smokers, and with an estimated 3-year lung cancer risk of >2% based on a prototype of a Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial lung cancer risk prediction model [15]. Enrolment occurred from September 2008 until December 2010. Demographic information was collected from participants and blood samples were drawn and stored at their baseline visit.

Spirometry and computed tomographic imaging

Yearly spirometry was performed based on American Thoracic Society/European Respiratory Society guidelines [16], with quality control conforming to criteria outlined by the Burden of Obstructive Lung Disease study [17]. Only pre-bronchodilator measurements were available for the entire cohort and thus were used in the analysis. Participants also underwent baseline low-dose computed tomography (CT) scanning of the chest. From these images, qualitative assessment of the extent of emphysema was performed using a method modified from Kazerooni *et al.* [18] and employed in the COPDGene Study [19]. Individual emphysema scores for each of the five lobes of the lung plus the lingula were assessed as a percentage of total lung volume, and a score of 0–4 was assigned, defined as follows: 0=absence of emphysema, 1=1–25% emphysema, 2=26–50% emphysema, 3=51–75% emphysema, and 4=76–100%

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emphysema. A total score was obtained by the summation of the six individual "lobe" scores (five lobes and lingula). Reading radiologists were unaware of spirometry values.

Pro-SFTPB measurements

Details describing the development of the pro-SFTPB assay and its ability to predict lung cancer in the Pan-Can cohort have been published previously [13]. Details of the assay are provided in the online supplementary material. Briefly, the sandwich pro-SFTPB ELISA was developed from mouse monoclonal antibodies against the N-terminus of pro-SFTPB. Anti-pro-SFTPB mouse monoclonal antibody (#464) was biotinylated with EZ-Link Sulfo-NHS-LC-Biotin (Thermo Scientific, Rockford, IL, USA) and used for incubation at 0.5 $\mu g \cdot mL^{-1}$. After washing, each well was incubated with streptavidin-horseradish peroxidase followed by incubation of colour reagents and addition of stop solution (R&D Systems, Minneapolis, MN, USA). The absorbance was measured at 450 nm with a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA) with a correction factor of 620 nm. The specificity of the pro-SFTPB assay was validated using mass spectrometry. For samples whose pro-SFTPB levels were below the level of detection, we assigned a value that was half the detection limit. Baseline plasma levels of pro-SFTPB were used for this analysis, with samples performed in duplicate.

Statistical analysis

Measurements of pro-SFTPB were first divided into quartiles to evaluate the relationship of pro-SFTPB to demographic and respiratory parameters. The baseline characteristics across the quartiles were compared using the Jonckheere–Terpstra trend test for continuous variables and the Cochran–Armitage test for dichotomous variables [20, 21]. Change in lung function over time was calculated as the change in FEV_1 (mL) per year over 1- and 2-year periods. Multivariable regression models with the outcome variable as the annual decline in FEV_1 ($mL \cdot year^{-1}$) over a 1-year and 2-year period were constructed using the following variables: age, body mass index (BMI), baseline FEV_1, current smoking status and sex. We performed the multivariate analysis using PROC MIXED to take into account the hierarchical structure of the covariates and the multiple measurements of FEV_1 over time within the same subjects. All analyses were performed using JMP or SAS statistical software (version 10.0; SAS Institute, Cary, NC, USA) and two-sided p-values <0.05 were considered significant.

Ethics and informed consent

This study was approved by the Clinical Research Ethics Board of the University of British Columbia (Vancouver, BC, Canada) and at each of the participating Pan-Can study sites. All participants in the study gave written informed consent.

Results

Baseline demographic characteristics

Plasma pro-SFTPB was measured in 2503 participants of the Pan-Can study. A flow diagram indicating patients enrolled and the reasons for exclusion from the study is shown in figure 1. Baseline characteristics for all subjects and for the quartiles of pro-SFTPB are shown in table 1. The mean±SE age of the cohort was 62.34±0.12 years and, although 38.2% of patients had quit smoking by the time of the study, heavy smoking habits were reported (mean±SE 54.09±0.47 pack-years). Patients in the quartiles with the highest pro-SFTPB levels were more likely to be men ($p=0.023$) and have a lower BMI ($p=0.006$). They were also more likely to be current smokers ($p<0.001$) and to have the greatest amount and duration of smoking, as given by pack-years ($p=0.005$).

Respiratory-related characteristics

Respiratory-related variables for all subjects and for the quartiles of pro-SFTPB are shown in table 2. Notably, those in the highest quartiles of pro-SFTPB levels had a lower FEV_1 % predicted ($p=0.007$) and FEV_1/FVC ratio ($p<0.001$), but there were no significant differences in FEV_1, FVC or FVC % predicted across the quartiles. Linear regression analysis of FEV_1 % predicted and FEV_1/FVC ratio as continuous variables also demonstrated a significant relationship with pro-SFTPB levels (fig. 2). Higher pro-SFTPB levels were seen in patients with more advanced Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages (p-trend <0.001) (fig. 3a), although there were no differences between patients with normal spirometry and those designated as GOLD stage unclassified (FEV_1/FVC $\geqslant$70% and FEV_1 <80% predicted). Chest CT imaging was available for qualitative scoring in 2467 patients. Higher CT emphysema scores, signifying greater extent of emphysema, were found in the highest quartiles of pro-SFTPB levels (p-trend <0.001) (fig. 3b).

For the 2220 subjects who had FEV_1 measurements until 1 year after enrolment and for the 1687 subjects who had FEV_1 measurements until 2 years after enrolment, a 1-year annual decline in FEV_1 and a 2-year

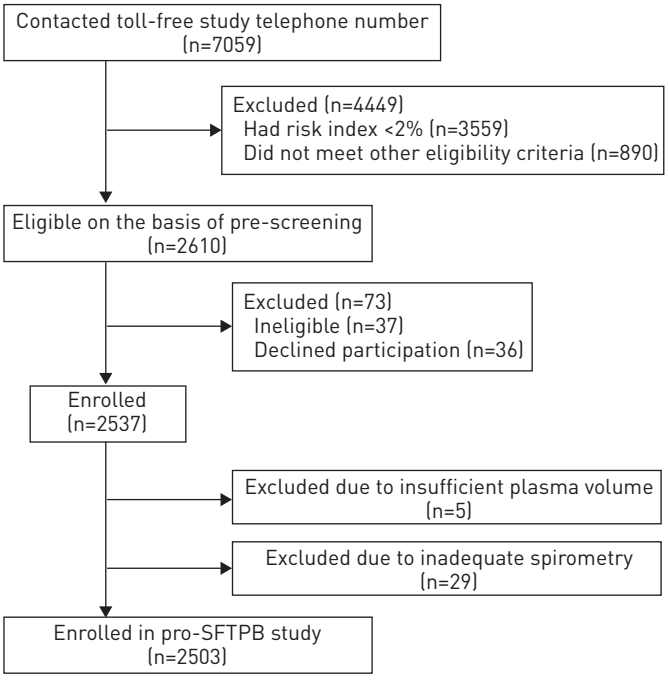

FIGURE 1 Flow diagram of patients included in and excluded from the Pan-Canadian Early Detection of Lung Cancer Study. Pro-SFTPB: pro-surfactant protein B.

TABLE 1 Baseline characteristics for all subjects and for pro-surfactant protein B (pro-SFTPB) quartiles

Category	All	Pro-SFTPB quartiles (ng·mL^{-1})				
		1 (<16.93)	2 (16.94–31.79)	3 (31.80–56.66)	4 (⩾56.67)	p-trend
Pro-SFTPB ng·mL^{-1}	45.53±0.92	11.10±0.15	23.69±0.17	42.86±0.29	104.58±2.29	
Patients	2503 (100)	626 (25)	626 (25)	626 (25)	625 (25)	
Age years	62.34±0.12	62.09±0.23	62.43±0.22	62.36±0.24	62.48±0.25	0.336
Female	1118 (44.67)	301 (48.08)	281 (44.89)	276 (44.09)	260 (41.60)	0.023
BMI kg·m^{-2}	26.50±0.09	26.98±0.18	26.42±0.18	26.43±0.17	26.18±0.16	0.006
Current smoker	1536 (61.81)	255 (41.00)	362 (58.29)	455 (72.68)	464 (75.33)	<0.001
Smoking pack-years	54.09±0.47	53.59±0.94	52.65±0.92	53.67±0.89	56.49±0.99	0.005

Data are presented as mean±SE or n (%), unless otherwise stated. BMI: body mass index.

TABLE 2 Respiratory variables for all subjects and for pro-surfactant protein B (pro-SFTPB) quartiles

Category	All	Pro-SFTPB quartiles (ng·mL^{-1})				
		1 (<16.93)	2 (16.94–31.79)	3 (31.80–56.66)	4 (⩾56.67)	p-trend
Pro-SFTPB ng·mL^{-1}	45.53±0.92	11.10±0.15	23.69±0.17	42.86±0.29	104.58±2.29	
Patients	2503 (100)	626 (25)	626 (25)	626 (25)	625 (25)	
CT emphysema total score	1.13±0.02	0.81±0.04	1.06±0.05	1.20±0.05	1.44±0.05	<0.001
FEV$_1$ L	2.44±0.01	2.45±0.03	2.47±0.03	2.44±0.03	2.40±0.03	0.174
FEV$_1$ % predicted	81.77±0.37	82.60±0.76	82.58±0.73	81.59±0.74	80.32±0.75	0.007
FVC L	3.57±0.02	3.53±0.04	3.61±0.04	3.59±0.04	3.55±0.04	0.880
FVC % predicted	92.44±0.35	92.39±0.72	93.03±0.67	92.72±0.68	91.63±0.72	0.349
FEV$_1$/FVC %	68.43±0.20	69.38±0.41	68.60±0.40	68.12±0.40	67.59±0.42	<0.001
1-year FEV$_1$ change$^{\#}$ mL·year^{-1}	−46.65±4.14	−30.01±7.85	−52.47±8.41	−48.81±8.44	−55.88±8.35	0.037
1-year FEV$_1$ change$^{\#}$ %	−1.46±0.19	−0.77±0.38	−1.70±0.38	−1.49±0.39	−1.91±0.37	0.034
2-year FEV$_1$ change¶ mL·year^{-1}	−40.37±2.70	−29.68±5.03	−42.90±4.92	−43.63±5.57	−46.14±8.35	0.005
2-year FEV$_1$ change¶ %	−2.85±0.24	−1.88±0.46	3.21±0.46	−3.01±0.51	−3.39±0.50	0.037

Data are presented as mean±SE or n (%), unless otherwise stated. CT: computed tomography; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity. $^{\#}$: available for 2220 subjects; ¶: available for 1687 subjects.

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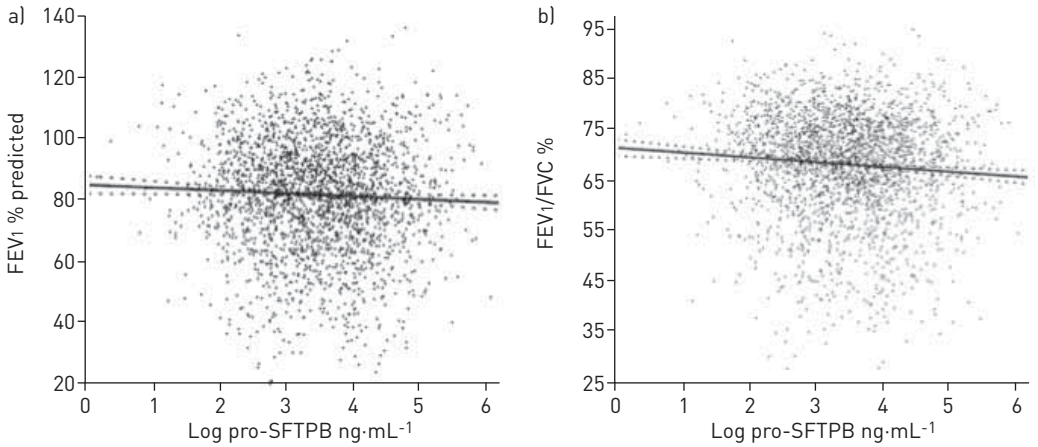

FIGURE 2 The univariate relationship between pro-surfactant protein B (pro-SFTPB) levels (logarithmic scale) and measures of airway obstruction by spirometry. The solid line represents the mean line and the dotted lines represent the 95% confidence intervals. a) Higher pro-SFTPB levels were associated with lower forced expiratory volume in 1 s (FEV_1) % predicted ($p=0.024$, $R^2=0.002$). b) Higher pro-SFTPB levels were associated with lower FEV_1/forced vital capacity (FVC) ratios ($p<0.001$, $R^2=0.007$).

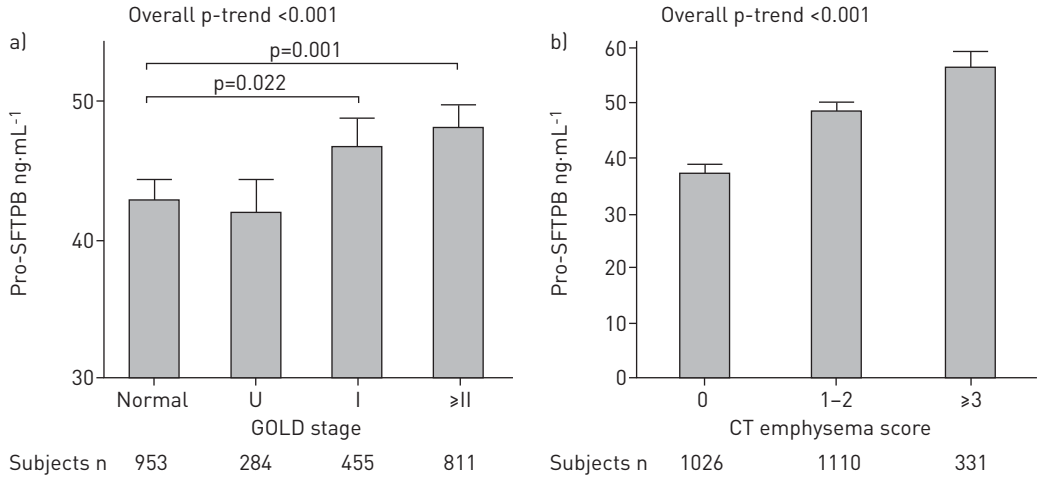

FIGURE 3 Pro-surfactant protein B (pro-SFTPB) levels in relation to measures of chronic obstructive pulmonary disease severity. Error bars represent SE. a) Pro-SFTPB levels for patients according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage classification. Higher pro-SFTPB levels were seen with more severe GOLD stages (overall p-trend <0.001). There was no significant difference in pro-SFTPB levels between normal and GOLD stage U individuals, or between GOLD stage I and ⩾II, by the Steel–Dwass test for multiple comparisons. U: GOLD unclassified stage (forced expiratory volume in 1 s (FEV_1)/forced vital capacity ≥70%, FEV_1 <80% predicted). b) Pro-SFTPB levels for patients according to computed tomography (CT) emphysema scores. Higher CT emphysema scores denote greater severity of disease. Higher pro-SFTPB levels were associated with higher CT emphysema scores (overall p-trend <0.001).

annual decline in FEV_1 were calculated, respectively. Prior to adjustment for confounding variables, patients in the highest pro-SFTPB quartiles had significantly greater 1-year annual FEV_1 declines, both in litres ($p=0.037$) and in percentage change from baseline ($p=0.034$). Similar trends were seen for 2-year percentage change in FEV_1 ($p=0.037$) and in FEV_1 in litres over 2 years ($p=0.005$). When analysed as continuous variables and after adjustment for confounding variables (age, sex, BMI, baseline FEV_1 (L), current smoking and pack-years of smoking), FEV_1 declines calculated over 1-year and 2-year periods were significantly greater in patients with the highest pro-SFTPB levels ($p=0.024$ and $p=0.004$, respectively) (fig. 4).

Multivariable linear regression models

Adjustments for significant covariates made little difference to the findings. Pro-SFTPB levels were significantly associated with rapid decline in lung function over 2 years (table 3). Higher pro-SFTPB levels were associated with severe CT emphysema (score ⩾4), higher pack-years of smoking, current smoking, older age and male sex (table 4).

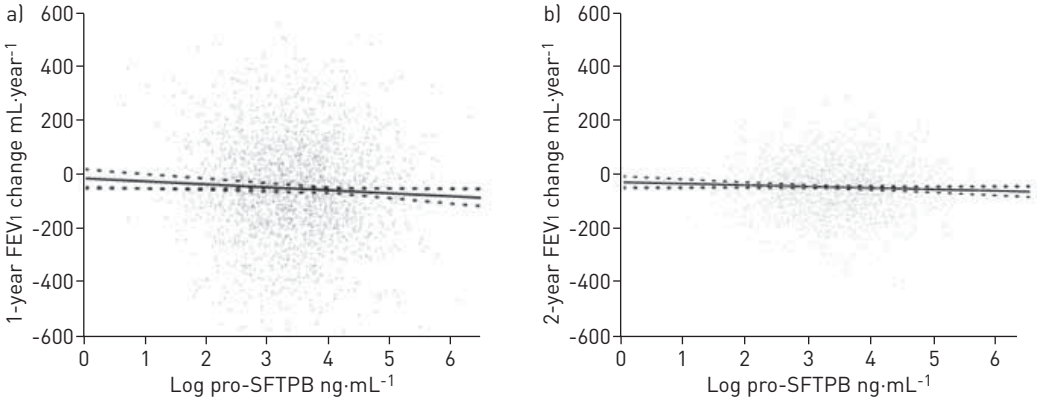

FIGURE 4 The adjusted relationship between pro-surfactant protein B (pro-SFTPB) levels (logarithmic scale) and the rate of decline in forced expiratory volume in 1 s (FEV_1) over a) 1 year and b) 2 years. Covariates adjusted for included FEV_1 (L), age, body mass index ($kg \cdot m^{-2}$), current smoking and sex. The solid line represents the mean line and the dotted lines represent the 95% confidence intervals. Higher pro-SFTPB levels were associated with a greater decline in FEV_1 in patients with a) 1-year spirometry values available ($p=0.006$, $R^2=0.059$) and b) 2-year spirometry values available ($p=0.004$, $R^2=0.076$).

TABLE 3 Linear regression models for forced expiratory volume in 1 s (FEV_1) change[#]

Parameter	β coefficient	SE	p-value	R^2
1-year FEV_1 change				
Age years	−2.510	0.745	<0.001	0.002
Female	29.772	5.159	<0.001	0.001
Smoking pack-years	−0.686	0.182	<0.001	0.001
Baseline FEV_1 L	−79.491	7.154	<0.001	0.036
Pro-SFTPB[¶]	−13.183	4.660	0.005	0.003
2-year FEV_1 change				
Age years	−1.414	0.487	0.004	0.001
Female	13.454	3.231	<0.001	0.003
Current smoking	15.877	5.730	0.006	0.010
BMI $kg \cdot m^{-2}$	1.508	0.627	0.016	0.007
Baseline FEV_1 L	−47.183	4.554	<0.001	0.053
Pro-SFTPB[¶]	−5.537	3.160	0.004[+]	0.004

The covariates included in the model were age, sex, current smoking status, body mass index (BMI; $kg \cdot m^{-2}$), baseline FEV_1 (L) and pro-surfactant protein B (pro-SFTPB) levels. Combined R^2 values for 1-year and 2-year FEV_1 change were 0.058 and 0.078, respectively. [#]: $mL \cdot year^{-1}$; [¶]: logarithmic scale; [+]: p-value based on PROC MIXED.

TABLE 4 Clinical and physiological factors associated with pro-surfactant protein B levels[#]

Parameter	β coefficient	SE	p-value	R^2
CT emphysema score ⩾4	0.132	0.040	0.001	0.006
Smoking pack-years	0.003	<0.001	<0.001	0.002
Current smoking	0.263	0.018	<0.001	0.074
Age years	0.014	0.003	<0.001	0.001
Male	0.033	0.017	0.049	0.003

Variables included in the model included baseline forced expiratory volume in 1 s (FEV_1), body mass index (BMI), smoking pack-years, current smoking, age, computed tomography (CT) emphysema score and sex. Baseline FEV_1 and BMI were removed from the final model. The combined R^2 value was 0.096. [#]: logarithmic scale.

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Discussion

The most novel finding in this study was that circulating levels of pro-SFTPB, a highly lung-specific protein, were associated with increased severity of airflow limitation and most importantly with rapid progression of COPD over 2 years in a large population of current and ex-smokers. While caution should be applied to clinical application, due to the wide variation in levels of pro-SFTPB, there appears to be a threshold effect whereby subjects in the second quartile of pro-SFTPB levels had markedly faster lung function decline than subjects in the first quartile. Clinical utility may be tied to this threshold level. We had previously shown that higher levels of circulating pro-SFTPB were associated with another important smoking-related lung disease, lung cancer [13]. Together, these data indicate that pro-SFTPB may be a very promising biomarker of two major smoking-related disorders: COPD and lung cancer.

Superficially, our current results appear to be discordant with a previous study that interrogated the relationship between plasma pro-SFTPB levels and airway obstruction [22]. However, this latter study contained a small sample size and as such lacked the statistical power to adequately evaluate this relationship. Our data are consistent with previous investigations that have evaluated surfactants in plasma with lung function. Surfactant protein D, for instance, has been shown to be increased in patients with severe COPD [23]. SFTPB has higher specificity for lungs (as its source) than other hydrophilic surfactants such as surfactant A or D, making it a much more desirable plasma biomarker for COPD. Although the exact mechanism for the increased levels of surfactants in plasma of severe COPD patients is unknown, it is postulated that with greater severity of lung disease, increased lung permeability allows for the transit of surfactant into the bloodstream where it is then measured in elevated quantities. A similar mechanism has been proposed for SFTPB [24, 25]. We speculate that increased plasma levels of pro-SFTPB may be the result of translocation from the lung to the blood, with disease being associated with primary lung deficiency; further studies would be necessary to confirm this mechanism.

How pro-SFTPB may come to reflect severe COPD phenotypes is subject to some debate. Murine models completely deficient in SFTPB die soon after birth from respiratory failure [26] and humans with frameshift mutations in the SFTPB gene develop neonatal respiratory distress [27, 28]. Partial deficiencies in SFTPB may also be problematic. For instance, heterozygosity for the *SP-B* 121ins2 mutation in humans, in which production of SFTPB is reduced by half, has also been shown to be associated with reduced lung function and the presence of COPD [29]. Similarly, mice with partial deficiencies of SFTPB develop lung physiology reminiscent of COPD. Despite seemingly normal lung growth and histological appearance, these mice display increased air trapping and early small airway closure compared with control mice with full complements of SFTPB [30]. Upstream regulators of SFTPB gene expression may play a role in this process. Transcriptional regulation of SFTPB is performed by thyroid transcription factor 1 (TTF-1)/NK2 homeobox 1 (NKX2-1), levels of which fluctuate in response to pulmonary injury [31]. One known trigger for such injury is ceramide, a second messenger sphingolipid responsible for mediating apoptosis [32]. By inhibiting TTF-1/NKX2-1 DNA-binding activity, ceramide effectively decreases SFTPB protein levels. Incidentally, ceramide has also been implicated in the development of emphysema [33]. Intratracheal installation of ceramide into mice causes increases in alveolar diameter, a process that is prevented in mice whose *de novo* synthesis of ceramide is blocked.

While apoptosis is thought to be the mechanism by which this emphysema develops, an additional role may be played by SFTPB, particularly considering its role in mediating inflammatory insults. SFTPB deficiency in a transgenic murine model increases several measures of pulmonary inflammation, including bronchoalveolar lavage fluid cell number, macrophage and neutrophil migration, and levels of interleukin (IL)-6, IL-1β and macrophage inflammatory protein-2 [8]. This heightened inflammatory response is, furthermore, attenuated with the restoration of SFTPB. Similarly, SFTPB, unlike surfactant proteins A and C, may modulate the oxidative stress response of the lung. Specifically, *in vitro* models of alveolar macrophages cultured from rat lungs and incubated with SFTPB showed reduced nitric oxide levels in response to lipopolysaccharide [9]. Both the anti-inflammatory and anti-oxidative properties of SFTPB may work to protect the lung from the toxic effects of cigarette smoke.

Our analysis has several limitations that should be taken into consideration. First, our patient population was made up entirely of former and current smokers. Generalisation of our findings to nonsmoking populations must therefore be performed with caution. Although it is well established that COPD can occur in nonsmokers, the performance of the pro-SFTPB plasma assay in predicting lung function decline in this population remains unknown. Secondly, we cannot at the moment claim that pro-SFTPB levels can predict long-term outcomes, as we were only able to measure lung function decline through a 2-year period after enrolment. Future studies would need not only to assess the ability of pro-SFTPB levels to predict lung function changes over longer periods of time, but also to evaluate other clinically important measures, such as respiratory-related health status, rate of exacerbations, hospitalisations and mortality. Thirdly, the use of pre-bronchodilator rather than post-bronchodilator spirometry was also a limitation to the study, as the diagnosis of COPD is preferably made

through post-bronchodilator measurements. Finally, we did not measure other inflammatory biomarkers in this cohort, so whether pro-SFTPB is merely a marker for inflammation or whether pro-SFTPB performs favourably or unfavourably compared with other inflammatory biomarkers remains unknown.

While previous efforts to quantify SFTPB have been hampered by the technical limitations of assaying a hydrophobic molecule, a new ELISA method based on SFTPB's pro-form now allows for accurate and reproducible measurements. Its specificity for reflecting the lung in particular may give pro-SFTPB an advantage over nonspecific inflammatory biomarkers that have yet to show proven clinical utility. We demonstrate here that pro-SFTPB may reflect certain measures of COPD severity. Future investigation over extended periods of spirometric follow-up may help to clarify what role pro-SFTPB can play in the diagnosis and management of COPD.

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