Levels of Surfactant Proteins A and D and KL-6 Are Elevated in the Induced Sputum of Chronic Obstructive Pulmonary Disease Patients: A Sequential Sputum Analysis

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Key Words

Surfactant protein A • Surfactant protein D • KL-6 • Induced sputum • Sputum fraction

Abstract

Background: Recent clinical studies have suggested that serum surfactant protein (SP) A, SP-D and Krebs von den Lungen-6 (KL-6) are potential biomarkers for interstitial lung diseases. Serum levels of SP-A and SP-D have also been found to be elevated in chronic obstructive pulmonary disease (COPD), but their significance has not been evaluated or compared in induced sputum samples obtained directly from the airways. **Objective:** A sequential sputum analysis was conducted to assess the value of SP-A, SP-D and KL-6 levels in COPD. **Methods:** The study material consisted of induced sputum samples from 61 subjects, 28 with COPD and 33 with prolonged cough (cough lasting >3 weeks and normal spirometry). Sputum was collected in 3 fractions (3 periods of 5 min each). Sputum levels of these proteins were measured, and receiver operating characteristic curve analysis was carried out to evaluate the sensitivity, specificity and area under the curve (AUC) for each fraction. **Results:** The levels of SP-A, SP-D and KL-6 were higher in patients with

COPD than in those with prolonged cough in each of the fractions. Sputum levels of these proteins correlated inversely with obstruction and positively with ageing, smoking history, sputum macrophages and eosinophils. Sputum fractionation had a relatively minor effect on the levels and AUC of these proteins. **Conclusion:** Sequential sputum analysis from 3 consecutive fractions indicated a significant difference in the levels of SP-A, SP-D and KL-6 between COPD and prolonged cough. However, sputum fractionation itself had a relatively minor effect on the levels of these proteins.

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Introduction

Collection of induced sputum is a nearly noninvasive technique for sampling of the airway secretions in chronic obstructive pulmonary disease (COPD). Induction also helps to achieve an adequate sample from the airways from those patients who are not able to produce sputum spontaneously. We and others have used induced sputum in COPD research to study the inflammatory profile, oxidant markers and potential biomarkers for COPD by both hypothesis-driven and proteomic methodologies

[1–8]. However, several studies have reported alterations in and problems with the cellular and biochemical compositions of sputum specimens during the induction procedure [9–12]. Very little is known about how inflammatory mediators or potential COPD biomarkers vary in induced sputum during the induction procedure.

Surfactant protein (SP) A, SP-D and Krebs von den Lungen-6 (KL-6) have been widely investigated in the circulating blood. All these proteins have been postulated to represent biomarkers for interstitial lung diseases, especially for idiopathic pulmonary fibrosis, though recent studies have revealed elevation of serum SP-A also in COPD [13, 14]. However, many results in serum and bronchoalveolar lavage fluid have been controversial [15, 16]. Recently, we performed a lung tissue proteomic study on subjects with COPD. This proteomic screening indicated that SP-A was elevated in mild to very severe COPD, and the result was confirmed in induced sputum specimens by Western blotting [17]. In addition to SP-A, other studies have shown that in advanced COPD, the serum level of SP-D is negatively associated with the forced expiratory volume in 1 s (FEV₁), and its increase over a 3-month period is associated with worsening of the health status of the patients [18, 19]. Those studies suggested that SP-D may be useful in identifying individuals who are at increased risk of experiencing exacerbations of COPD [20]. Generally, SP-A, SP-D or KL-6 have been investigated from the circulating blood, but they have not been studied separately in induced sputum supernatants of patients with COPD. No study has evaluated the effect of sputum fractionation on the levels of these proteins in COPD.

The present study was undertaken to (1) assess whether sputum levels of SP-A, SP-D and KL-6 can be used to differentiate subjects with COPD from those with prolonged cough and normal spirometry and (2) identify possible changes in the induced sputum levels of these proteins between sequentially collected sputum specimens.

Materials and Methods

Subjects

Altogether, 61 subjects were included in the study, 28 of whom had COPD [Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I–III disease]. The diagnosis of COPD was defined according to the GOLD criteria, i.e. FEV₁/forced vital capacity (FVC) <70% and bronchodilatation effect <12% [21, 22]. The medications used in our subjects included short-acting β_2 -agonists in all subjects, long-acting β_2 -agonists in 6 subjects, inhaled corticosteroids in 7 subjects and anticholinergics in 10 subjects. No subjects were receiving systemic corticosteroid therapy. A further 33 subjects with prolonged cough were included as controls.

Table 1. Characteristics of the subjects

	COPD	Prolonged cough
Subjects, n	28	33
Females/males, n	2/26	25/8***
Age, years	70.4 $\pm$ 2.0	52.6 $\pm$ 2.3***
BMI	21.9 $\pm$ 0.6	23.6 $\pm$ 0.6
Smoking history		
Current/former/never, n	10/18/0	3/7/23
Pack-year history	65.6 $\pm$ 8.9	6.5 $\pm$ 2.3***
Spirometry		
FVC, liters	3.18 $\pm$ 0.12	3.01 $\pm$ 0.14
FEV ₁ , liters	1.72 $\pm$ 0.10	2.43 $\pm$ 0.12***
FEV ₁ , % predicted	72.8 $\pm$ 4.5	104.4 $\pm$ 3.0***
FEV ₁ to FVC ratio	0.55 $\pm$ 0.03	0.81 $\pm$ 0.02***

Data are shown as numbers of patients or means $\pm$ SEM, as appropriate. *** $p < 0.001$ compared to COPD patients (Mann-Whitney U test or Fisher's exact test).

The diagnosis of prolonged cough lasting >3 weeks was made according to the Japanese cough guidelines [23]. Subjects with prolonged cough had no airway obstruction (postbronchodilator FEV₁/FVC >70%) and no significant reversibility (less than 12% reversibility for FEV₁ after 400 μ g of inhaled salbutamol) and did not fulfill asthma criteria. Four had allergic rhinitis and 1 had an atopic history. Prolonged cough resulted from cough-variant asthma-like symptoms in 11 subjects, sinobronchial syndrome in 5, gastroesophageal reflux disease in 3 and postinfectious cough in 14. The subjects were not allowed to smoke during the 12 h prior to sputum specimen collection. Symptom-free nonsmokers were excluded since they generally have difficulties to produce a representative sputum specimen. Moreover, in preliminary studies and in the study of Ohlmeier et al. [17], SP-A was not generally detectable in the sputum samples of nonsmokers. The Ethics Committee of the Hiroshima University Hospital approved the study. All patients received information about the trial and provided permission to use the samples. For detailed information, see table 1.

Lung Function Values

Spirometry (FEV₁, FVC and FEV₁ to FVC ratio) was performed for all subjects using a computed spirometer as previously described [24, 25]. The protocol for lung function measurements followed the American Thoracic Society recommendations [26].

Sputum Induction and Processing

Sputum specimens were obtained from subjects with COPD and prolonged cough at the Hiroshima University Hospital according to the guidelines of the European Respiratory Society's Task Force [27, 28]. In brief, a sputum induction procedure was conducted using 3–5% hypertonic saline given at 5-min intervals for a maximum of 15 min via an ultrasonic nebulizer (NE-U12, Omron Co., Kyoto, Japan) with an output of 3.0 ml/min. The concentration of hypertonic saline was changed during the procedure, starting with 3% and subsequently increasing up to 5%; the

Table 2. Characteristics of induced sputum

	COPD			Prolonged cough		
	5 min	10 min	15 min	5 min	10 min	15 min
Weight, mg	2.0 ± 0.3	3.1 ± 0.5	3.2 ± 0.4 ^b	2.2 ± 0.4	2.7 ± 0.4	3.5 ± 0.6 ^c
Total cell count, × 10 ⁵ cells/ml	26.7 ± 9.1	32.6 ± 15.2	49.1 ± 21.9	12.3 ± 2.7	15.4 ± 3.5	16.2 ± 2.7
Sputum neutrophils, %	87.5 ± 2.0	79.9 ± 3.4	74.4 ± 4.0 ^a	83.5 ± 3.5	75.1 ± 3.5	68.3 ± 4.4 ^c
Sputum macrophages, %	7.1 ± 1.6	14.8 ± 3.3	19.8 ± 4.1 ^a	10.3 ± 2.3	17.3 ± 2.6	24.6 ± 3.8 ^c
Sputum lymphocytes, %	3.0 ± 1.0	2.4 ± 0.9	2.2 ± 0.8	3.0 ± 0.7	3.9 ± 1.1	3.5 ± 0.8
Sputum eosinophils, %	2.3 ± 1.0	2.9 ± 1.3	3.8 ± 1.4	3.1 ± 1.9	3.9 ± 2.2	3.5 ± 1.9
Sputum neutrophils, × 10 ⁵ /g	9.7 ± 2.9	8.2 ± 2.8	13.0 ± 5.2	6.6 ± 1.5	5.0 ± 1.4	3.9 ± 0.7
Sputum macrophages, × 10 ⁵ /g	1.1 ± 0.6	0.9 ± 3.0	3.0 ± 1.6	0.6 ± 0.2	1.5 ± 0.9	1.1 ± 0.3
Sputum lymphocytes, × 10 ⁵ /g	0.18 ± 0.06	0.12 ± 0.04	0.41 ± 0.27	0.20 ± 0.07	0.34 ± 0.16	0.15 ± 0.03
Sputum eosinophils, × 10 ⁵ /g	0.15 ± 0.03	0.12 ± 0.04	0.53 ± 0.40	0.09 ± 0.03	0.10 ± 0.05	0.11 ± 0.04

Data are shown as means ± SEM. ^a $p < 0.01$, ^b $p < 0.001$ between 5-, 10- and 15-min samples obtained from subjects with COPD (repeated-measures ANOVA). ^c $p < 0.001$ between 5-, 10- and 15-min samples obtained from subjects with prolonged cough (repeated-measures ANOVA).

sputum samples produced after the first, second and third 5-min inhalation periods were processed separately [29, 30]. All sputum samples were processed immediately after induction. The entire sample was weighed and mixed with an equal volume of 0.1% diethylenetriamine (Sigma-Aldrich, St. Louis, Mo., USA) in phosphate-buffered saline solution. The suspension was centrifuged, and the supernatants were immediately stored at -80°C for later assay. The cell pellets from the sputum centrifugation were resuspended, the suspension was air-dried and stained with Diff Quick (Sysmex, Kobe, Japan) and the viabilities and total numbers of the cells were determined. The percentages of macrophages, neutrophils, lymphocytes and eosinophils were obtained by counting >200 nonsquamous cells. Sputum samples containing >20% squamous cells and cell viability <70% were considered inadequate and excluded from the further analyses. The cell counting was performed by an experienced observer blinded to the clinical characteristics of the subjects.

Measurement of SP-A, SP-D and KL-6 Levels in the Induced Sputum

Sputum levels of SP-A and SP-D were measured by commercially available EIA/ELISA kits (SP-A test: Kokusai-F kit, Sysmex; SP-D kit: Yamasa EIA kit, Yamasa Co., Chiba, Japan) as described previously [13]. KL-6 levels were measured by sandwich-type electrochemiluminescence immunoassay using a Picolumi 8220 Analyzer (Sanko Junyaku, Tokyo, Japan) as described elsewhere [31]. The detection limits for serum SP-A, SP-D and KL-6 were 1 ng/ml, 17.2 ng/ml and 3 U/ml, respectively.

Statistical Analysis

Data are presented as means ± SEM. The data were analyzed with a statistical software package (SPSS for Windows, version 15.0, SPSS Inc., Chicago, Ill., USA), and $p < 0.05$ was considered to indicate a significant difference. The comparison between 5-, 10- and 15-min samples was performed by repeated-measures analysis of variance (ANOVA). Differences between 2 groups were ana-

lyzed using the Mann-Whitney U test or Fisher's exact test. Correlations between the variables were calculated by Spearman's rank correlation coefficient. Sputum levels of SP-A, SP-D and KL-6 were further analyzed for their predictive capability to distinguish patients with COPD from those with prolonged cough according to receiver operating characteristic (ROC) curves.

Results

Subject Characteristics

The clinical characteristics of the subjects are shown in table 1. In the COPD group, there were 13 subjects with stage I, 8 with stage II and 7 with stage III COPD according to the GOLD classification. The total number of subjects in the prolonged cough group was 33. Some characteristics differed significantly between the COPD and prolonged cough groups. Younger age, female gender and nonsmokers were more prevalent in the prolonged cough group than in the COPD group.

Sputum Cells

The weight and total cell counts of the sputum samples obtained at the 3 induction points, i.e. early (5 min), intermediate (10 min) and late (15 min), are presented in table 2. During induction, the weight of the sputum specimens in both groups of subjects increased significantly ($p < 0.001$ and $p < 0.001$, by repeated-measures ANOVA). The percentage of neutrophils decreased significantly during induction in the COPD and prolonged cough groups ($p = 0.002$ and $p < 0.001$, respectively), whereas the

Fig. 1. Sputum levels of SP-A (a), SP-D (b) and KL-6 (c) in 5-, 10- and 15-min induced sputum obtained from 28 subjects with COPD (◆) and 33 with prolonged cough (PC). Prolonged cough was related to cough-variant asthma-like symptoms in 11 subjects (●) and to other causes in 22 (○). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Mann-Whitney U test).

percentage of macrophages increased ($p = 0.003$ and $p < 0.001$, respectively). However, no corresponding changes during induction could be seen in the absolute numbers of cells in either group (table 2). When the composition of induced sputum was compared between the subjects with COPD and those with prolonged cough, neither the differential cell count percentages nor absolute cell numbers of the sputum differed between the two groups.

SP-A, SP-D and KL-6 Levels in Induced Sputum

The levels of SP-A, SP-D and KL-6 in the different fractions of induced sputum are shown in figure 1. Levels of SP-A, SP-D and KL-6 did not change during induction in any group (by repeated-measures ANOVA). The levels of SP-A in the 5-, 10- and 15-min sputum fractions were higher in the COPD than in the prolonged cough group

($p = 0.002$, $p = 0.002$ and $p = 0.005$, respectively, by Mann-Whitney U test; fig. 1a). The levels of SP-D in each fraction were also higher in the COPD than in the prolonged cough group ($p = 0.014$, $p = 0.002$ and $p = 0.006$, respectively; fig. 1b). The KL-6 levels remained stable during induction, but were higher in COPD patients compared to prolonged cough patients in all 3 fractions ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively; fig. 1c).

ROC Curve Analysis

ROC curve analysis was carried out to evaluate the sensitivity, specificity and diagnostic accuracy of sputum levels of SP-A, SP-D and KL-6 in each fraction. The area under the curve (AUC) was quite similar in each fraction, suggesting that the sputum fractionation had a relatively minor effect on the levels of these proteins (fig. 2).

Fig. 1. Sputum levels of KL-6 (**c**) in 5-, 10- and 15-min induced sputum obtained from 28 subjects with COPD (◆) and 33 with prolonged cough (PC). Prolonged cough was related to cough-variant asthma-like symptoms in 11 subjects (●) and to other causes in 22 (○). *** $p < 0.001$ (Mann-Whitney U test).

Correlation of Sputum Levels of SP-A, SP-D and KL-6 with Clinical Variables, Lung Function Values and Sputum Cell Counts

The levels of SP-A, SP-D and KL-6 correlated positively with age and smoking status and inversely with several lung function values (table 3). The levels of SP-A in the 5-min sputum fraction were higher in males than in females ($p = 0.011$, Mann-Whitney U test), and the levels of KL-6 in each fraction were higher in males than in females ($p = 0.009$, $p = 0.022$ and $p = 0.036$, respectively). Significant correlations were seen between the FEV₁ to FVC ratio and induced sputum levels for SP-A in the 5-, 10- and 15-min fractions ($p = 0.007$, $p = 0.013$ and $p = 0.020$, respectively, by Spearman's rank correlation coefficient), for SP-D in the 10-min fraction ($p = 0.010$) and

for KL-6 in each of the 3 fractions ($p = 0.002$, $p = 0.003$ and $p = 0.008$, respectively). FEV₁ and FEV₁% predicted also correlated with induced sputum levels of SP-A and KL-6 in the 10-min fraction, whereas they did not correlate with those in the 5- and 15-min fractions. As shown in table 4, the levels of SP-A, SP-D and KL-6 correlated positively with macrophage and eosinophil numbers.

Reproducibility of SP-A, SP-D and KL-6 Analyses

Sputum levels of SP-A, SP-D and KL-6 were assayed in two separate runs to validate the reproducibility of the analyses from frozen samples. The two separate runs for SP-A, SP-D and KL-6 correlated highly significantly ($r = 0.953$ and $p < 0.001$, $r = 0.948$ and $p < 0.001$, and $r = 0.971$ and $p < 0.001$, respectively, by Spearman's rank correlation coefficient).

Discussion

The present study supports the hypothesis that the levels of SP-A, SP-D and KL-6 are elevated in COPD when analyzed from induced sputum specimens. Sputum levels of all these proteins could differentiate subjects with COPD from those with prolonged cough. In particular, the levels of SP-A, SP-D and KL-6 correlated inversely with obstruction and positively with ageing and smoking history. Overall, sputum fractionation had a relatively minor effect on the levels and AUC of these proteins. This is the first sequential sputum study investigating the levels of SP-A, SP-D and KL-6 in patients with COPD.

SP-A and SP-D belong to a subgroup of the C-type lectin superfamily (molecular weight: 26–36 and 43 kDa, respectively), and they have been implicated in the regulation of pulmonary host defense and inflammation [32, 33]. KL-6 is a high-molecular-weight (>200 kDa) mucin-like glycoprotein which has been classified as human MUC1 mucin [34, 35]. Serum/plasma SP-A, SP-D and KL-6 have been reported to represent sensitive markers for lung diseases, to the extent that in Japan they are used in clinical practice during the identification of idiopathic pulmonary fibrosis and other types of interstitial lung diseases. However, there are several problems in studies where SP-A has been evaluated from the circulating blood such as its elevation also in smokers, subjects with COPD or bronchiectasis and even in pulmonary thromboembolism [13–20]. It is likely that sputum samples that are derived directly from the lung would offer better insight, especially into the pathogenesis of chronic airway and/or other parenchymal lung diseases. A novel aspect of the present study

Fig. 2. ROC curves of SP-A, SP-D and KL-6 during induction (5-, 10- and 15-min samples) in subjects with COPD and prolonged cough. Values in parentheses indicate 95% confidence intervals for the AUC.

is that it represents a comparative study of SP-A, SP-D and KL-6 in induced sputum samples using sensitive and specific EIA/ELISA analysis for each marker.

It has been suggested that small-sized SP-A and/or SP-D leak more easily in parenchymal lung damage compared to the high-molecular-weight KL-6 so that more extensive enhancement of alveolar-capillary permeability is needed for the elevation of circulating KL-6 [36, 37]. Previous immunohistochemical analyses have revealed that SP-A and SP-D are prominently expressed in type II alveolar pneumocytes [38] and that KL-6 is expressed in type II alveolar pneumocytes and in bronchiolar epithe-

lial and bronchial gland cells [39]. Other studies have detected SP-A also in the bronchiolar, tracheal, sinus and middle ear epithelium [17, 40–43]. This study indicates that sputum levels of SP-A, SP-D and KL-6 were elevated in the induced sputum of COPD patients compared to patients with prolonged cough. This may simply be due to the alveolar destruction, but it may also reflect the wide expression of these proteins in both the large and small airways of the diseased human lung. Since one typical feature in COPD is mucus production, more studies are needed to unravel the expression and cell-specific distribution of KL-6 in COPD and in its subphenotypes.

Table 3. Correlations between the sputum levels of SP-A, SP-D and KL-6 and the clinical parameters

	SP-A			SP-D			KL-6		
	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Age	0.351*	0.167	0.145	0.374*	0.337*	0.351**	0.575***	0.457***	0.459***
Pack-years	0.324*	0.201	0.181	0.290	0.263	0.140	0.436**	0.379**	0.372**
FVC	0.096	-0.056	0.131	-0.026	-0.075	0.015	0.040	-0.111	0.072
FEV ₁	-0.046	-0.268*	-0.073	-0.046	-0.269*	-0.119	-0.146	-0.332*	-0.134
FEV ₁ % predicted	-0.040	-0.360**	-0.091	0.064	-0.281*	0.004	-0.061	-0.344*	-0.081
FEV ₁ to FVC ratio	-0.392**	-0.324*	-0.308*	-0.276	-0.343**	-0.219	-0.450**	-0.394**	-0.349**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (correlation between variables, by Spearman's rank correlation coefficient).

Table 4. Correlations between the sputum levels of SP-A, SP-D and KL-6 and sputum cell counts

	SP-A			SP-D			KL-6		
	5 min	10 min	15 min	5 min	10 min	15 min	5 min	10 min	15 min
Sputum neutrophils (%)	-0.227	-0.104	-0.135	-0.370*	-0.117	-0.187	-0.368*	-0.190	-0.328*
Sputum macrophages (%)	0.438**	0.228	0.225	0.474**	0.057	0.180	0.531**	0.278*	0.318*
Sputum lymphocytes (%)	-0.157	0.174	-0.095	-0.212	0.039	-0.097	-0.173	0.064	-0.114
Sputum eosinophils (%)	0.160	-0.006	-0.014	0.336*	0.343*	0.170	0.346*	0.199	0.198
Sputum neutrophils ($\times 10^5/g$)	0.282	0.129	0.247	0.243	0.049	0.131	0.141	0.123	0.184
Sputum macrophages ($\times 10^5/g$)	0.480**	0.310*	0.367**	0.477**	0.172	0.329*	0.481**	0.384**	0.479**
Sputum lymphocytes ($\times 10^5/g$)	0.038	0.209	0.020	-0.048	0.018	0.021	-0.047	0.123	0.074
Sputum eosinophils ($\times 10^5/g$)	0.249	0.029	0.060	0.469**	0.330*	0.221	0.453**	0.242	0.261

* $p < 0.05$, ** $p < 0.01$ (correlation between variables, by Spearman's rank correlation coefficient).

One major goal of the present study was to evaluate whether sputum fractionation itself has any major effects on the levels of these proteins. In this study, the percentage of neutrophils was higher in the early fraction than in the later fraction, whereas the percentage of macrophages increased towards the end of the induction. These results are in agreement with those of several previous investigations [9–12] and suggest that different parts of the respiratory tract are sampled into different fractions during induction. There is also evidence that induced sputum better reflects the central rather than the lower and peripheral airways [44–46]. One study on 12 subjects with mild and moderate asthma revealed that sputum levels of mucin-like glycoprotein are higher in the early fraction than in the later fraction, whereas sputum levels of SP-A appeared to be higher in the later fractions [11]. In agreement with these results, sputum SP-A levels in our subjects with prolonged cough showed an increasing trend towards the end of the sputum induction. Overall, sputum

levels of all these proteins in each fraction resulted in a very similar ROC curve for the differentiation of the patients with COPD from the subjects with prolonged cough.

Although promising results were obtained, this study has limitations. One limitation is the low number of patients and thus the poor statistical power. The subjects with COPD were older than those with prolonged cough. The levels of sputum SP-A, SP-D and KL-6 increased with age, though the change was observed in both nonsmokers and smokers. More males had been included in the COPD group and more females in the prolonged cough group; there was also a significant difference between males and females, especially with regard to sputum levels of KL-6. The levels of SP-A, SP-D and KL-6 in the induced sputum supernatants of COPD patients were higher than in those of the prolonged cough patients in both nonsmokers and smokers. However, it is possible that smoking alone can increase these markers in sputum through an acute stress reaction or due to the minimal lung injury. There was also

individual variability in the sputum levels of these proteins, which may be related to multiple reasons, from sputum production in general to dithiothreitol treatment, which in turn can interfere with immunoassays by reducing the antigen-antibody binding reaction. Certain technical factors, including the duration of the inhalation procedure and the concentration of saline solution, might have influenced the procedure, but previous studies have indicated that different saline concentrations do not significantly affect total cell count and differential cell counts [47] and that shorter inhalation times (e.g. 15–20 min) appear to have similar success rates and feasibility to the longer inhalation times (30 min) [27]. Since saliva generally contains much lower levels of mucins [48], possible contamination with saliva may have contributed to the final results obtained. In spite of these limitations, the main results of our study were straightforward and significant. However, more studies will be required in age-, sex- and smoking-history-matched subjects with various airway diseases compared to smoking and nonsmoking controls.

Even though induced sputum is relatively reproducible in COPD [49, 50], a large and prospective study, including also non-Asian subjects, will be required to evaluate the utility of sputum levels of SP-A, SP-D and KL-6 in COPD. The validation and definition of the specificity of these proteins in various chronic airway diseases will

require further studies, especially in various phenotypes of COPD (airway vs. emphysema predominance) [22], allergic asthma, intrinsic-type asthma and asthma in smokers. A prospective follow-up of this nature is currently ongoing in a non-Asian (Finnish) cohort which contains unselected patients with asthma and COPD [51].

Conclusion

Sequential sputum analysis of induced sputum from 3 consecutive fractions demonstrated a significant difference between subjects with COPD and prolonged cough with regard to the sputum levels of SP-A, SP-D and KL-6. Sputum fractionation had a relatively minor effect on the levels of these proteins.

Financial Disclosure and Conflicts of Interest

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