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Serum surfactant protein-A, but not surfactant protein-D or KL-6, can predict preclinical lung damage induced by smoking

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Abstract

Serum surfactant protein (SP)-A offers a useful clinical marker for interstitial lung disease (ILD). However, SP-A is occasionally elevated in non-ILD pulmonary patients. The present study was conducted to investigate factors that affect serum SP-A levels in respiratory medicine. Serum SP-A, serum SP-D, serum Klebs von den Lungen (KL)-6 and pulmonary function tests were evaluated in 929 patients (current smokers, $n=255$; ex-smokers, $n=242$; never-smokers, $n=432$) without ILD or pulmonary alveolar proteinosis. Serum SP-A was significantly higher in current smokers than in never- or ex-smokers ($p<0.01$ and $p<0.05$, respectively). Serum SP-A was significantly higher in chronic obstructive pulmonary disease (COPD) and pulmonary thromboembolism than in other diseases ($p<0.01$). Serum SP-A correlated positively with amount of smoking ($p<0.01$) and negatively with forced expiratory volume in 1 s/forced vital capacity ($p<0.05$). Serum SP-D and KL-6 were unaffected by smoking. Smoking should be taken into account when evaluating serum SP-A levels, and different baseline levels of serum SP-A should be established for smokers and non-smokers. Serum SP-A may also represent a useful marker for predicting COPD in the preclinical stage.

Keywords: *Surfactant protein-A (SP-A), smoking, chronic obstructive pulmonary disease (COPD), pulmonary thromboembolism*

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Introduction

Surfactant protein (SP)-A, SP-D and Klebs von den Lungen (KL)-6 are easily measured in serum and now widely used to evaluate patients in respiratory clinics (Kuroki et al. 1998, Ohinishi et al. 2002, Takahashi et al. 2006). These serum markers are well researched and sensitive for interstitial lung disease (ILD) activity and early disease detection. However, serum SP-A is elevated in pulmonary alveolar proteinosis, bacterial pneumonia, tuberculosis and pulmonary adenocarcinoma, and changes in these three markers are not always consistent or affected in parallel in disease courses. Opinions in the literature vary from 'increased leakage of SP-A and SP-D compared with KL-6 during alveolar injury' (Kuroki 1998, Takahashi 2000a) to 'KL-6 is the

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best serum marker for ILD' (Ohinishi et al. 2002). Research is continuing to investigate how to distinguish the significance of each marker (Abe & Takahashi 2001) and which marker demonstrates clinical superiority for the diagnosis and investigation of various disease activities. In our clinical experience, serum SP-A is often elevated in patients other than those with ILD or bacterial pneumonia, particularly in current smokers. Conversely, Greene et al. (2002) reported that smoking does not affect serum SP-A. Smoking effects on SP-A levels in bronchoalveolar lavage fluid have been investigated (Takahashi 2000a, Betsuyaku et al. 2004) but research to date using large serum samples has been insufficient. We therefore intended to determine definitively whether or not smoking affects serum SP-A levels and what other lung diseases might influence serum SP-A in a large cohort of respiratory patients without ILD.

Methods

Patients

A total of 929 patients (452 men, 477 women) with respiratory diseases, excluding ILD, pulmonary alveolar proteinosis, pneumonia or adenocarcinoma, were enrolled in this study. Patients who had a history of prolonged exposure to inhalation of dusty air in occupational and/or environmental conditions were also excluded (Berthoin et al. 2004). Based on smoking history, patients who had quit smoking $\geq$6 months previously were defined as ex-smokers. Amount of smoking was quantified using the Brinkman index (BI: number of cigarettes consumed per day, multiplied by years of smoking). Patients who had quit smoking $<$6 months previously were excluded. Mean age of patients was 59.6$\pm$10.2 years (range 19–86 years). Fifty-nine patients were in their 20s or younger, 109 were in their 30s, 104 were in their 40s, 216 were in their 50s, 283 were in their 60s, and 158 were in their 70s or older. All cases were diagnosed based on radiological (including computed tomography), physiological (including pulmonary function tests), bacteriological and/or histological examinations. The underlying pathology was sarcoidosis in 223 (mean age 48.1 years), neoplastic disease in 191 (65.6 years), chronic obstructive pulmonary disease (COPD) in 118 (67.2 years), non-tuberculous mycobacterial disease (NTM) in 86 (61.3 years), bronchial asthma in 68 (50.3 years), mediastinal disease in 48 (60.1 years), bronchiectasis in 36 (63.8 years), pleural disease in 35 (59.5 years), pulmonary thromboembolism in 9 (53.9 years) and other disease in 115 patients. The remaining pathologies included chronic cough, bloody sputum, congenital anomalies, post-infectious sequelae and miscellaneous pulmonary diseases. No patients were receiving administration of oral steroids or nicotine replacements.

All patients provided informed consent to participate. The Institutional Review Board of the Department of Medicine at the National Defense Medical College approved all study protocols.

Evaluation

SP-A was assayed using a commercially available electrochemiluminescence immunoassay kit (SP-A test; Sysmex, Kobe, Japan) for frozen serum (Takahashi et al. 2006). This test has undergone thorough regulatory assessment and is approved as a clinically validated test. The reference value is $\leq$43.8 ng ml^{-1}. Serum KL-6 (Sanko Junyaku, Kobe, Japan), serum SP-D (Yamasa Shoyu, Chiba, Japan) and pulmonary

function tests (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC and carbon monoxide diffusion capacity) were also measured. Serial measurements were performed in 114 patients. SP-A, SP-D and KL-6 were measured by a commercial laboratory (SRL, Tokyo, Japan).

Intra-assay coefficients of variation ranged from 4.5% to 6.15% and interassay coefficients of variation ranged from 4.8% to 7.18% (Sysmex and SRL). Five concentration levels were used as standards for assays. No drifts of standard curves with time were seen.

Statistical analysis

Data are displayed as mean $\pm$ standard deviation. Statistical analysis including group comparison, correlation, linear regression, and paired *t*-test were performed using EXCEL Tahenryo-Kaiseki version 5.0 (Esumi Co. Tokyo, Japan). The level of statistical significance for all tests was $p < 0.05$.

Results

Mean serum SP-A was 35.1 ± 20.8 ng ml^{-1} in all 929 patients. Mean level was 35.7 ± 21.3 ng ml^{-1} in men and 34.9 ± 20.4 ng ml^{-1} in women, with no differences apparent between sexes. Serum SP-A level tended to increase in older patients, but no significant differences were apparent (data not shown). In current smokers ($n = 255$), ex-smokers ($n = 242$) and never-smokers ($n = 432$), serum SP-A levels were 47.2 ± 27.4 ng ml^{-1}, 35.8 ± 15.6 ng ml^{-1} and 32.8 ± 13.9 ng ml^{-1}, respectively (Figure 1). Although significant differences were observed between current smokers and ex-smokers ($p < 0.05$) and between current smokers and never-smokers ($p < 0.01$), the difference between ex-smokers and never-smokers was not significant.

Mean serum SP-A level was 30.7 ± 20.7 ng ml^{-1} in sarcoidosis, 40.2 ± 32.5 ng ml^{-1} in neoplastic disease, 68.8 ± 26.2 ng ml^{-1} in COPD, 39.9 ± 15.8 ng ml^{-1} in NTM, 31.3 ± 12.5 ng ml^{-1} in bronchial asthma, 35.1 ± 11.1 ng ml^{-1} in mediastinal

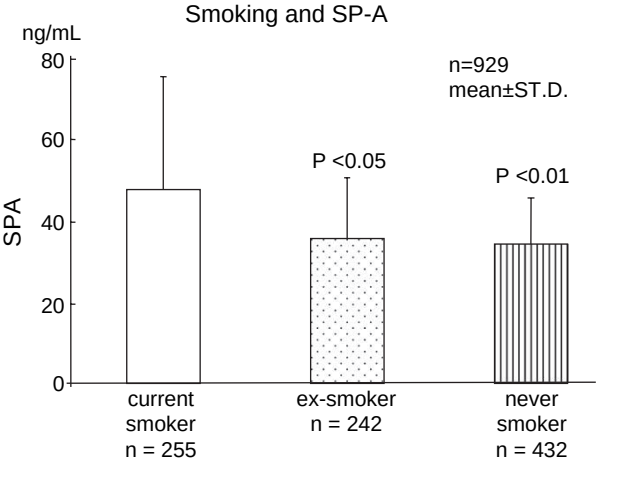

Figure 1. Serum SP-A in current, ex- and never-smokers. SP-A in current smokers (47.2 ± 27.4 ng ml^{-1}) was significantly higher than those in ex-smokers (35.8 ± 15.6 ng ml^{-1}) and never-smokers (32.8 ± 13.9 ng ml^{-1}). Wilcoxon rank test.

disease, 36.4 ± 15.6 ng ml^{-1} in bronchiectasis, 31.0 ± 19.8 ng ml^{-1} in pleural disease and 73.1 ± 35.6 ng ml^{-1} in pulmonary thromboembolism. Serum SP-A was significantly higher in COPD and pulmonary thromboembolism than in other diseases ($p < 0.01$; Figure 2).

In the 802 patients with conditions other than COPD or pulmonary thromboembolism, serum SP-A was 42.4 ± 19.9 ng ml^{-1} in current smokers ($n = 187$), 34.2 ± 15.6 ng ml^{-1} in ex-smokers ($n = 192$) and 32.3 ± 16.5 ng ml^{-1} in never-smokers ($n = 423$). Serum SP-A was significantly higher in current smokers than in ex- or never-smokers ($p < 0.001$). In COPD, serum SP-A was significantly higher in current smokers ($n = 68$; 75.6 ± 28.7 ng ml^{-1}) than in ex-smokers ($n = 50$; 47.8 ± 17.6 ng ml^{-1}; $p < 0.01$).

In 48 current smokers who successfully quit smoking, serum SP-A was measured a second time, 9.2 ± 3.9 months later. Serum SP-A after quitting smoking (43.9 ± 23.1 ng ml^{-1}) was significantly lower than before quitting smoking (57.4 ± 19.8 ng ml^{-1}; $p < 0.01$) (Figure 3). In 46 never-smokers, serum SP-A was also measured a second time, 10.7 ± 5.6 months later, and no significant difference was evident between first and second measurements.

Next, the correlation coefficient between SP-A and amount of smoking (BI) was examined by linear regression analysis. In current smokers ($n = 255$), a significant positive correlation was seen between BI and serum SP-A ($r = 0.39$, $p < 0.01$) (Figure 4). The slope was 0.049 ± 0.0024 (95% confidence interval (CI) 0.044–0.053). No significant correlations were found in ex-smokers ($n = 242$, $r = 0.23$) or COPD ($r = 0.21$).

For pulmonary function tests, current smokers displayed a significant negative correlation between FEV_1/FVC ratio and serum SP-A ($r = -0.31$, $p < 0.05$)

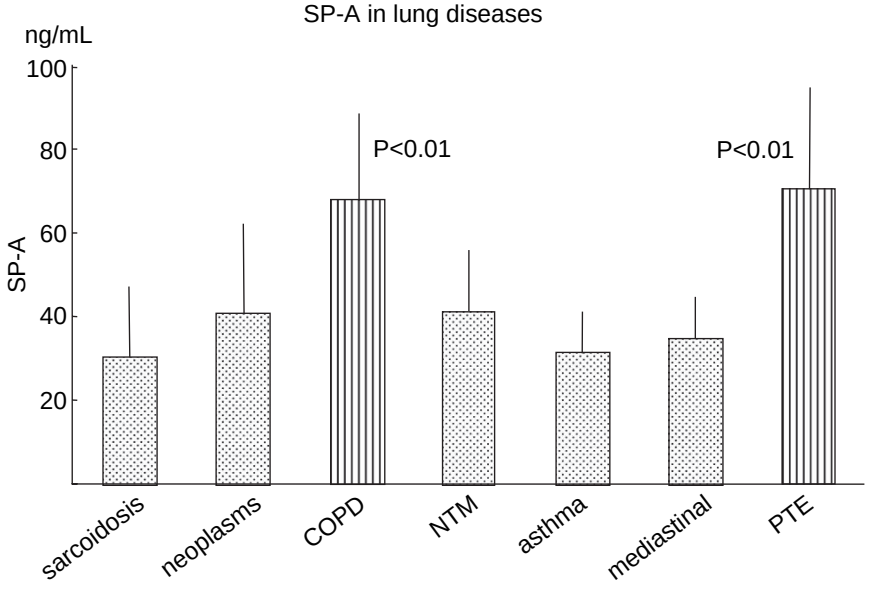

Figure 2. Serum SP-A in various lung diseases. Serum SP-A in chronic obstructive pulmonary disease (COPD) and pulmonary thromboembolism (PTE) was significantly higher than in the other disorders (Kruskal–Wallis test, $p < 0.01$). NTM, non-tuberculous mycobacterial infection.

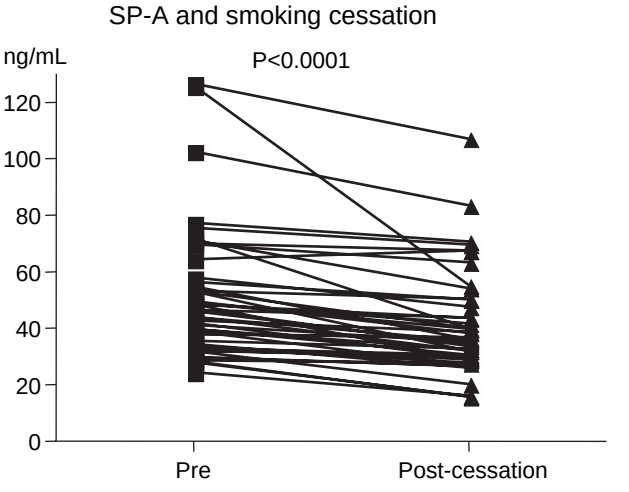

Figure 3. Serum SP-A before and after smoking cessation in 48 patients. SP-A was significantly decreased after smoking cessation (paired *t*-test, $p < 0.01$). The interval between evaluations was 9.2 ± 3.9 months.

(Figure 5). The slope was -1.27 ± 0.17 (95% CI, -1.59 to -0.94). The correlation coefficient was -0.22 in ex-smokers and -0.30 in COPD ($p < 0.05$). No correlations were observed between SP-A and other functional measurements, FVC/predicted FVC ($r = -0.13$), FEV_1 ($r = -0.15$) or diffusion capacity ($r = -0.12$).

Serum SP-D ($n = 824$) and KL-6 ($n = 818$) levels were also analyzed (Table I). Smoking had no significant effects on SP-D or KL-6 levels.

Discussion

SP-A is a glycoprotein that is a member of the pulmonary collectins; it is synthesized by alveolar epithelial type II and Clara cells and plays an important role in lung defence mechanisms (McCormack & Whitsett 2002). Serum SP-A, SP-D and KL-6 levels have been widely used for over 10 years in the clinical evaluation of ILD (Kuroki

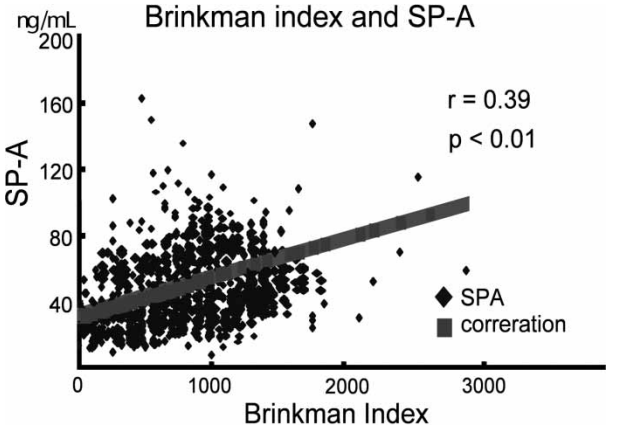

Figure 4. Correlation between Brinkman index and serum SP-A. Brinkman index is (amounts of tobacco per day) × (years in smoking). Correlation coefficient (r) was 0.39 (simple linear regression, $p < 0.01$).

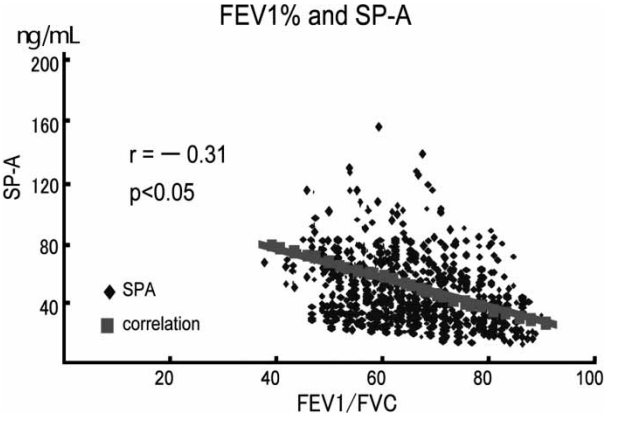

Figure 5. Correlation between forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) and serum SP-A in current smoker. Correlation coefficient (r) was 0.31 (simple linear regression, $p < 0.05$).

et al. 1998, Takahashi et al. 2000a, Abe & Takahashi 2001, Ohnishi et al. 2002, Takahashi et al. 2006). However, differences in the clinical significance of these three markers are not completely understood (Takahashi et al. 2000a, Ohnishi et al. 2002, Al-Salmi et al. 2005).

In our clinical experience, COPD patients in whom the complication of ILD has been ruled out by computed tomography show higher serum SP-A, and smokers display serial elevations in serum SP-A. However, Greene et al. (2002) have reported that smoking has no effect on serum SP-A and Mutti et al. (2006) described similar results. Conversely, Robin et al. (2002) described significantly higher serum SP-A levels in smokers, although data from a large sample have not previously been collected regarding the effects of smoking on serum SP-A. Furthermore, SP-A levels vary in other pulmonary disorders. One study found that SP-A was only rarely elevated in COPD, whereas another study in COPD patients reported higher levels. The number of patients included in similar studies has not been sufficient (Abe & Takahashi 2001, Rabe et al. 2007).

Our results from a large number of cases showed that serum SP-A level was significantly higher in current smokers than in ex- or never-smokers. Also, in COPD, which is a disease caused by smoking (Rabe et al. 2007), serum SP-A levels were higher than in other diseases (Figures 1–3). In addition, serum SP-A levels were even higher in current smokers with COPD than in ex-smokers with COPD ($p < 0.01$). Serial measurements showed that serum SP-A decreased in patients who had quit smoking (Figure 3). These findings demonstrate that smoking increases serum SP-A. However, serum SP-D and KL-6 levels were unaffected by smoking (Table I). This suggests smoking as one reason for the discrepancy in diseases between these three

Table I. Serum SP-D and KL-6 in respiratory patients. Serum SP-D and KL-6 were not significantly affected by the smoking habit of patients.

	SP-D (ng ml^{-1}) ($n = 824$)	KL-6 (U ml^{-1}) ($n = 818$)
Current smokers	69.4 ± 25.5	341.9 ± 155.4
Ex-smokers	65.8 ± 27.3	332.4 ± 146.6
Never-smokers	67.2 ± 28.1	329.9 ± 167.2

serum markers. Ishii et al. (2003) reported that serum SP-A was significantly higher in usual interstitial pneumonia (UIP) than in non-specific interstitial pneumonia (NSIP). However, only 16% of NSIP patients were current smokers, whereas 42% of UIP patients were current smokers, so smoking may have contributed to some of the difference in SP-A levels (Ishii et al. 2003).

As the second point of our results, significant positive correlations were seen between SP-A and amount of smoking, whereas negative correlations existed between SP-A and FEV_1/FVC. Regression coefficients (r) were insufficient, but the large number of patients included in this study was sufficient to obtain significant p-values. Serum SP-A might be a clue to detect smokers likely to develop COPD before respiratory symptoms or physiological functional disturbances become apparent.

Patients in this study were not age-matched, even though aging might affect SP-A levels (Ueda et al. 2000, Betsuyaki et al. 2004). In general, COPD is a disease that develops in the older aged, while bronchial asthma and sarcoidosis develop in the younger generation. The generation characteristics of the disease made compensating for age-matching groups difficult. As significant correlations could not be detected between age and SP-A in COPD or between age and SP-A in any patients (data not shown), aging therefore might not influence SP-A in patients.

Type II alveolar epithelial cell hyperplasia, increases in cell number, alveolar epithelial injury and hyperpermeability between the epithelial–endothelial barrier cause increases in serum SP-A (Takahashi et al. 2000b, McCormack 1998). When smoking causes alveolar epithelial injury, SP-A, with the lowest molecular weight of about 30 kDa, leaks into the bloodstream before SP-D and KL-6 (McCormack 1998, Takahashi et al. 2000b). This explains why serum SP-A is the first marker to increase. As alveolar injury due to smoking is not severe, the rise in SP-A is a gradual and cumulative process. One-point assay of SP-A is thus not a sensitive discriminating factor for separating smokers from non-smokers.

We also observed increased SP-A levels in pulmonary thromboembolism. Thromboembolism causes alveolar ischaemia and epithelial and endothelial damage, eventually resulting in serum SP-A elevation. Our results suggest that serum SP-A may also represent a useful and valuable marker in diagnosing pulmonary thromboembolism and assessing response to treatment.

We emphasize the need to establish higher baseline levels of SP-A for smokers. New baseline levels should be determined using healthy volunteers who currently smoke. In addition to being a marker for ILD, serum SP-A can also detect smoking-related lung damage. In current smokers, serum SP-A may be elevated before pulmonary function tests show obstructive ventilatory changes. Although smokers do not always develop clinical COPD (Celli et al. 2004), serum SP-A levels might help to identify asymptomatic smokers at higher risk of developing COPD (Cheng et al. 2000, Behera et al. 2005). Further studies should investigate whether serial changes in serum SP-A indicate a precursor state to COPD and whether SP-A could be expected to detect smokers likely to develop COPD before respiratory symptoms become apparent.

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