Factors Influencing the Measurement of Plasma/Serum Surfactant Protein D Levels by ELISA

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Abstract

Background: Extensive variations in human surfactant protein D (SP-D) levels in circulation as measured by ELISA exist in the published literature. In order to determine the source of these variations, factors influencing the measurement by ELISA were explored.

Materials and Methods: Peripheral blood from healthy individuals was collected into various vacutainers during the same blood draw. Recombinant SP-D was diluted into different matrices and used for a standard curve. Samples were analyzed by capture ELISA using one of two distinct detection antibodies.

Results: The type of matrix had some effects on detection of recombinant SP-D. The type of anticoagulant used and dilution factor had very little effect, except for in plasma collected in EDTA vacutainers. The extent of variation in published values seemed to be due to the ELISA configuration employed, and, in agreement with this, we found that by switching the detection antibody, there was a 50% decrease in the extrapolated SP-D value of serum and plasma samples. Storage of samples resulted in slight changes in measured SP-D levels.

Conclusions: The ELISA configuration employed to measure circulating levels of SP-D has a significant effect on the extrapolated values. In both configurations tested, the use of EDTA as a coagulant resulted in inconsistent values, and we, therefore, suggest the avoidance of this anticoagulant when assaying for SP-D by ELISA. While the demonstrated effects of several factors on measurement of SP-D may not account for all the disparities amongst the previous studies, they stress that variations in methodologies for measuring the same protein can result in very inconsistent results.

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Introduction

Surfactant protein D (SP-D) is a pulmonary collectin involved in regulation of inflammation, innate immune defense, and surfactant homeostasis. It is expressed by Clara cells and alveolar type II cells in the lung. SP-D has a multimeric structure which gives it the ability to agglutinate pathogens, as well as aid in the clearance of apoptotic cells, cellular debris, and foreign particles in the lung [reviewed in [1]].

Circulating levels of SP-D have been examined for their potential use as a biomarker in various diseases including dermatitis [2,3], acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) [4–13], periodontitis [14], interstitial pulmonary fibrosis (IPF) [10,12,15–23], chronic obstructive pulmonary disease (COPD) [15,24–36], emphysema [37], cystic fibrosis (CF) [15,38,39], coronary disease [40,41], sclerosis [42–46], cancer [47,48], sarcoidosis [21,49], allergies [28,50–52], rheumatoid arthritis [53,54], and respiratory infections [18,55–60]. SP-D levels have also been proposed to correlate with genetic elements [61–63], body mass index (BMI) [64–68], age [65], circadian rhythm [69], and with particle exposure [70,71] and cigarette smoking habits [25,27,28,31,37,72–77]. In addition, there have been studies examining the levels of SP-D in subjects with Turner syndrome [78], paraquat intoxication [79], swimming in variably treated waters [80], lung transplant patients [81], patients undergoing neurosurgical operations [82], drowning victims [83], polyomysitis/dermatomyositis [84], dementia [85], lupus [86], and sleep apnea [87].

Similarly to CC-16 and KL-6, SP-D is thought to be a marker of pulmonary leak into the vasculature [88], and therefore alveolar destruction would result in an increase in levels of these pulmonary proteins in the blood. However, protein levels in lung do not always correlate with protein levels in blood [35], suggesting the
possibility of alternative mechanisms affecting SP-D levels in circulation.

Various commercially available kits and non-commercially available ELISA configurations have been used to compare SP-D levels in plasma and serum from normal, healthy controls and the various disease states described above. Interestingly, there is a very substantial discrepancy between the reported values of the healthy control populations between studies, as well as in the magnitude of the range of values in this population. While the ELISA configuration used to measure SP-D seemed to have a large impact on the values reported, there are significant variations in the healthy control SP-D levels and range amongst reports using the same configuration. The purpose of this study is to determine factors affecting the measurement of SP-D by ELISA that may, therefore, explain the variations of serum and plasma SP-D levels reported in the published literature.

Materials and Methods

Study subjects, peripheral blood collection, and processing

All human studies were approved by the University of Alabama at Birmingham Institutional Review Board for Human Use with all subjects providing written consent. Peripheral blood was collected from healthy volunteers by venipuncture into serum, heparin sulfate, K$_2$EDTA, and sodium citrate vacutainers (BD Biosciences) during a single draw. Samples were kept at room temperature until blood in the serum tube was coagulated. Afterwards, samples were centrifuged at 400xg for 10 minutes to separate blood cells from serum or plasma. Samples were either directly aliquoted and stored at −80°C or given an additional round of centrifugation at 3000xg for 10 minutes to separate platelets from serum or plasma. All serum and plasma sample values depicted in figures were free of platelets. All experiments had 6 samples per group except for the experiments depicted in Figures 2 and 4.

Measurement of SP-D concentration by ELISA and data analysis

Mouse antihuman SP-D capture antibody, biotinylated mouse antihuman SP-D detection antibody, streptavidin conjugated to horse radish peroxidase, and recombinant human SP-D standard were purchased as a kit from R&D Systems (Catalog # DY1920) and used according to the manufacturer’s protocol. For experiments testing the effects of different matrices on the detection of recombinant human SP-D, a concentrated stock was made in PBS with 1% BSA from separately purchased recombinant human SP-D expressed in NSO cells (R&D Systems, Catalog # 1920-SP-050). For studies comparing detection antibodies, polyclonal goat antihuman SP-D antibody (R&D systems, Catalog # AF1920) was used instead with a monoclonal mouse antigoat conjugated to horse radish peroxidase (Sigma) used as a secondary. All sample values were extrapolated from a second order polynomial curve fit of 9 concentrations of the standard (two-fold dilutions with a high concentration of 40 ng/mL) diluted in 1% BSA PBS. Statistical analyses were performed as described in the figure legends using Prism 5 (GraphPad Software), and all reported p values are two-tailed. All error bars represent standard deviation.

Results

Variations in previously reported SP-D levels

In order to determine the mean and range of SP-D levels in the serum/plasma of normal, healthy individuals, we performed a non-exhaustive search of the literature which revealed more than 60 publications in which these values were described. Interestingly, an unexpected amount of variation on these reported values was observed. When grouping according to the ELISA configuration used, one configuration consistently resulted in significantly higher values than the two other configurations for which multiple references were obtained (Figure 1a). For each configuration, a substantial range of means/medians was observed in the healthy control population. In addition to the variations seen in the averages, the spectrum of values in all populations ranged from 55.9 pg/mL [22] to 3.9653 µg/mL [89], representing a more than 70,000 fold difference. When examining the range of values seen in healthy subjects from each individual study and grouping according to the ELISA configuration used, a large variation in range was observed (Figure 1b). Overall, the study with the smallest range for any population (as a percentage of the average) reported a 95% confidence interval of 95.7–109.0 [54], while the study containing the population with the largest range reported a standard deviation of 327% of the mean value [42].

Based on the above observations, we can infer that the largest difference in the measured levels of SP-D is due to the ELISA configuration employed, but that there are still significant differences between the averages of healthy individuals from studies using the same configurations. In order to compare the results of various studies in another manner, we examined the fold change from healthy populations for the four diseases/conditions for which a substantial number of publications were available (IPF, CF, cigarette smoking, and systemic sclerosis). This would allow us to control for ELISA configurations employed, technical protocol followed for the ELISA as well as for sample collection and processing, and any differences in the populations (as controls have been appropriately matched). The expectation was that the fold change from healthy would be very similar for each disease/condition. However, large differences in the extent of this change were observed (Figure 1c).

Influence of matrix on detection of recombinant SP-D

In order to determine if the matrix used to generate the serial dilutions of recombinant human standard (rhSP-D) had any effects upon measurement, we compared values produced by dilutions in PBS, solutions of BSA (in PBS), and solutions of FBS (diluted in PBS) (Figure 2). At higher concentrations of protein (i.e. 5% BSA, 50% FBS, and 100% FBS), a decrease in the amount of rhSP-D detected was observed, with the amount measured in samples containing 10 ng/mL rhSP-D being the most variable of any samples measured in this assay. Using the manufacturer’s recommended matrix (1% BSA) produced results very similar to a 10% FBS matrix, with both giving very consistent measurements and the greatest values. The values for PBS matrix gave similarly consistent measurements, but at a slightly lower value. This effect may be due to adsorption of rhSP-D without a carrier protein to the tubes used for serial dilutions [90]. Serum Matrix (Millipore), which had background levels of SP-D, also inhibited detection of recombinant SP-D at higher concentrations. All further standard curves were established by serial dilutions of recombinant SP-D in 1% BSA in PBS.

Influence of anticoagulant on extrapolated SP-D level

One factor that was found to differ between studies and could, therefore, be a source of variation of reported healthy population values, was the type of anticoagulant (or lack thereof) in the collection container. When blood was simultaneously collected in various vacutainers, serum and heparin plasma gave similar measurements of SP-D, while citrate plasma gave values...
significantly lower than serum values (Figure 3). EDTA plasma gave the most inconsistent results, and values were also significantly lower than serum values.

Influence of calcium on detection of SP-D by ELISA

In order to determine if calcium concentration in the sample had a significant effect upon the measurement of SP-D, rhSP-D was assayed in serially diluted CaCl₂ or EDTA. The detection of rhSP-D was only slightly effected by the changes in calcium concentration in the sample (Figure 4a). Additionally, we examined the effects of calcium concentration in the human samples by adding calcium to EDTA plasma samples and by adding EDTA to serum samples. While reconstituting the free calcium in the EDTA samples had little effect, the addition of EDTA to the serum samples had a dramatic effect that was inconsistent amongst the patient samples.

While many ELISAs employ the use of PBS lacking divalent cations as a buffer during the detection of antibody-captured antigens, the ELISA configuration employed by Holmskov et al. uses a Tris-buffered saline solution containing 5 mM calcium chloride, as the monoclonal detection antibody binds to SP-D in the presence of calcium [58]. In addition, it was previously demonstrated that SP-D has the ability to bind to various immunoglobulins in a calcium-dependent manner [91]. In order to determine the effects of calcium in the context of SP-D detection by ELISA, ELISAs were performed with antibodies diluted in buffer with or without calcium. We examined these effects on both recombinant SP-D and SP-D in plasma with either the ELISA kit’s detection antibody or a polyclonal goat anti-SP-D antibody produced by the same manufacturer. In all cases but one, the addition of 5 mM calcium to the dilution and wash buffer resulted in a small (~17%) but significant increase in SP-D concentration relative to detection in the absence of calcium (Figure 4b). It is important to note that with the kit reagents, since the relative increase in detection of recombinant SP-D and SP-D in plasma in the presence of calcium is very similar, the extrapolated SP-D concentration in plasma when using a standard curve of recombinant SP-D should not significantly change. Interestingly, while the inclusion of calcium increased the recognition of native SP-D by the polyclonal antibody, it had no effect on the detection of recombinant SP-D by this antibody. Although it is beyond the scope of this study, future work will explore whether this effect is due to an increase in antibody recognition of antigen, increase in non-specific binding of antibodies by captured SP-D, or an increase through another mechanism.

Influence of detection antibody on extrapolated SP-D level

Given that some of the variation seen in the published literature might be explained by the use of different antibodies, we detected SP-D in serum and plasma samples using either the ELISA kit’s detection antibody or the polyclonal goat anti-SP-D antibody. While there is no significant difference between detection by the

Figure 1. Published values for [SP-D] in the blood. a) A substantial amount of variation in the average [SP-D] in the serum/plasma of healthy control population exists between studies using the same or different ELISA configurations. Three configurations (Yamasa [12,13,17–19,21,25,28,38,43–45,49,50,59,61,77,84,89], BioVendor [14,31–36,46,52,56,64,65,72,75,76,79,80], and Holmskov et al. [3,39,53–55,58,62,66,69,70,78,85,86]) were compared. b) The range of healthy control [SP-D] greatly varied from study to study. Values shown are either median and interquartile range (IQR), median and 95% confidence interval (95%), or mean and standard deviation (SD). c) The calculated fold increase from the average healthy [SP-D] and average [SP-D] during IPF [12,16–19,21], CF [15,38,39], cigarette smoking [28,31,72–75], or sclerosis [42–44,46] was different between publications. An asterisk (*) denotes p<0.001 by one-way ANOVA with Tukey’s multiple comparison test.

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kit antibody versus the polyclonal goat antibody with regard to the type of collection tube used, overall, there is a ~50% reduction in the extrapolated value produced by detection with the polyclonal goat antibody compared to the kit antibody (Figure 5). In both cases, the same capture antibody was employed, and it is therefore possible that using a different capture antibody could have a similar effect on varying the extrapolated SP-D concentration in the sample.

Influence of sample dilution on extrapolated SP-D level

Another factor that fluctuated from study to study was whether the sample was assayed neat or the sample was prediluted (and the amount the sample was diluted). Given this fact and that diluting recombinant SP-D into 100% FBS had some influence in the detection of said SP-D, we compared SP-D values detected in undiluted and diluted samples. There was a very minimal difference between values produced by undiluted and 10 fold diluted serum, citrate plasma, and heparin plasma (Figure 6). Ten fold diluted EDTA plasma, however, produced a significantly higher extrapolated SP-D value when compared to the same sample undiluted. This same effect was seen when the polyclonal goat antibody tested above was used for detection (data not shown).

Influence of storage condition on extrapolated SP-D level

While the most common processing technique involved separating the blood cells (but not platelets) from serum and plasma and storing this sample at ~80°C until assayed, some variations in processing and storage were present. To address this, we assayed serum and heparin plasma without platelets immediately after processing or after storage at 4°C, ~20°C, or ~80°C (Figure 7); there were no significant differences between conditions. Additionally, there were

Figure 2. Use of various diluents for the recombinant SP-D standard. A 1 µg/mL recombinant SP-D stock was diluted 1:99 in various matrices and then serially diluted 2 fold in the same matrix. Mean and standard deviation for three independent experiments are shown. An asterisk (*) denotes values are significantly different (p<0.01) from both the 1% BSA and 10% FBS values by one-way ANOVA using Tukey’s multiple comparison test.
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Figure 3. Detection of SP-D in serum and plasma collected using various anticoagulants. a) SP-D concentrations were measured in samples collected into four different vacutainers during a single blood draw. b) Measured values of samples were normalized to the SP-D concentration in serum for each patient in order to compare the effect of the anticoagulant on the measured SP-D concentration. An asterisk (*) denotes values are significantly different by Wilcoxon signed rank test (for EDTA, p = 0.0156, and for Citrate, p = 0.0078).
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only subtle differences between a sample stored at $-20^\circ$C and $-80^\circ$C, and depending on whether the sample went through a freeze/thaw (FT) cycle, contained platelets or not, or was spun after thawing to separate any precipitates/debris (data not shown). SP-D concentrations of samples stored at $-20^\circ$C for two weeks were not different from the same samples at one week (data not shown).

**Discussion**

This study provides experimental evidence that variations in the anticoagulant used and ELISA configuration can have dramatic effects upon the measured SP-D level. Storage and processing of samples as well as diluent used for the standard have minor effects on the level of SP-D extrapolated by ELISA. Although these results may partially explain the variations seen in reported SP-D values in the blood, it is important to note the caveats associated with this review of the literature. For the comparison of healthy control groups amongst various studies, it is possible that differences in ethnic background and/or geographical location from which subjects were recruited may contribute to the
Accuracy of SP-D measurements is critically important to the validation of this protein as a biomarker in pulmonary disease. Standardization of sample processing and storage, including the avoidance of EDTA as an anticoagulant, is necessary to ensure consistent results. Although absolute values may vary greatly due to the ELISA configuration employed, relative differences in SP-D concentrations amongst various disease groups should be consistent.
Author Contributions
Conceived and designed the experiments: PEB. Performed the experiments: PEB. Analyzed the data: PEB. Contributed reagents/materials/analysis tools: PEB AG. Wrote the paper: PEB AG.

References
1. Kishore U, Greenough TJ, Waters P, Shrive AK, Ghai R, et al. (2006) Surfactant proteins SP-A and SP-D: structure, function and receptors. Mol Immunol 43: 1293–1313.

2. Maeda M, Ishikawa H, Aoyama Y, Kinajima Y (2001) Surfactant protein D (SP-D) in idiopathic pulmonary fibrosis. Am J Dermatol 59: 467–474.

3. Hohwy T, Otka J, Madsen J, Soerensen G, Nielsen O, et al. (2001) Surfactant protein D in atopic dermatitis and psoriasis. Exp Dermatol 10: 168–174.

4. Egan MD, Parsins P, Matthews MA, Ware L, Greene K, et al. (2003) Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax 58: 983–988.

5. Watkins TR, Rubenfeld GD, Martin TR, Nester TA, Caldwell E, et al. (2008) Effects of leukoreduced blood on acute lung injury after trauma: a randomized controlled trial. Crit Care Med 36: 1495–1499.

6. Todd DA, Marsh MJ, George A, Henderson NG, Barr H, et al. (2010) Surfactant phospholipid, surfactant proteins, and inflammatory markers during acute lung injury in children. Crit Care 14: 212–219.

7. Ware LB, Koyama T, Billheimer DD, Wu W, Bernard GR, et al. (2010) Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 137: 280–286.

8. Derdema RM, Royakkers AA, Haitsma JJ, Zhang H, Slutsky AS, et al. (2010) Plasma levels of surfactant protein D and KL-6 for evaluation of lung injury in critically ill mechanically ventilated patients. BMC Med 10: 6.

9. Collard HR, Galiff CS, Wolters PJ, Song JW, Hong SB, et al. (2010) Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 299: L5–L7.

10. Ware LB, Koyama T, Zhou Z, Jans DR, Wackersham N, et al. (2013) Biomarkers of lung epithelial injury and inflammation distinguish severe sepsis patients with acute respiratory distress syndrome. Crit Care 17: R253.

11. Greene KE, King TP, Jr, Kuroki Y, Bucher-Barrett B, Hummingleck GW, et al. (2002) Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. Eur Respir J 19: 439–446.

12. Greene KE, Wright JR, Steinberg KP, Ruzinski JT, Caldwell E, et al. (1999) Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. Am J Respir Crit Care Med 160: 1843–1850.

13. Glas J, Beynon B, Bacchin L, Buchenbiller J, Manoliu M, et al. (2000) Increased plasma concentration of surfactant protein D in chronic periodontitis independent of SFTTD genotype: potential role as a biomarker. Tissue Antigens 72: 21–28.

14. Sims MW, Bees MF, Ahya VN, Kwast SM, Sims KD, et al. (2011) Effect of single vs bilateral lung transplantation on plasma surfactant protein D levels in idiopathic pulmonary fibrosis. Chest 139: 496–474.

15. Nagao H, Takahashi H, Kuroki Y, Honda Y, Nagaia A, et al. (1997) Enzyme-linked immunosorbent assay using Fab′2 fragment for the detection of human pulmonary surfactant protein D in sera. Clin Chim Acta 266: 157–171.

16. Ohnishi H, Yokoyama A, Kondo K, Hamada H, Abe M, et al. (2002) Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1 as serum markers for interstitial lung diseases. Am J Respir Crit Care Med 165: 378–381.

17. Kuwano K, Maeyama T, Inoshima I, Ninomiya K, Hagimoto N, et al. (2002) Increased circulating levels of soluble Fas ligand are correlated with disease activity in patients with fibrosing lung diseases. Respirology 7: 15–21.

18. Takahashi H, Shiriatori M, Kanai A, Chiba H, Kuroki Y, et al. (2006) Monitoring markers of disease activity for interstitial lung diseases with serum proteins SP-A and -D. Respirology 11: 53–58.

19. Lin PC, Chen YC, Chang SC (2008) Clinical importance of bronchoalveolar lavage fluid and blood cytokines, surfactant protein D, and Kerbs von Langen 6 antigen in idiopathic pulmonary alveolar proteinosis. Mayo Clin Proc 83: 1344–1349.

20. Ando M, Mizukami E, Ito T, Hiroshi S, Nourski SL, et al. (2010) Significance of serum vascular endothelial growth factor level in patients with idiopathic pulmonary fibrosis. Lung 188: 247–252.

21. Ichiyao H, Ichikado K, Yamashita A, Iyonaga K, Sakamoto O, et al. (2012) Pneumoocyte biomarkers KL-6 and surfactant protein D reflect the distinct findings of high-resolution computed tomography in non-specific interstitial pneumonia. Respiration 83: 190–197.

22. Takacova R, McWilliam A, Lam S, Sin DD (2010) Integrating lung and plasma expression of pneumo-proteins in developing biomarkers in COPD: a case study of surfactant protein D. Med Sci Monit 16: CR140–CR44.

23. Baumets W, Mazzur T, Loschelainen N, Nieminen P, et al. (2011) Ageing and smoking contribute to plasma surfactant proteins and protease imbalance with correlations to airway obstruction. BMC Pulm Med 11: 19.

24. Celi BR, Locantore N, Yates J, Tal-Singer R, Miller BE, et al. (2012) Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 185: 1065–1072.

25. Ueno T, Lam S, Coxson H, Man SF, Sin DD (2013) Budesonide/formoterol enhances the expression of pro Surfactant Protein-B in lungs of COPD patients. PLoS One 8: e38381.

26. Mutri A, Corradi M, Goldoni M, Vettori MV, Bernard A, et al. (2006) Enaloeh steel elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma. Chest 129: 1288–1297.

27. Sin DD, Leung R, Gan WQ, Man SP (2007) Circulating surfactant protein D as a potential lung-specific biomarker of health outcomes in COPD: a pilot study. BMC Pulm Med 7: 13.

28. Sin DD, Man SF, Marcinuk DD, Ford G, Fitzgerald M, et al. (2008) The effects of fluicaizone with or without salmeterol on systemic biomarkers of inflammation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 177: 1207–1214.

29. Lomas DA, Silverman EK, Edwards JD, Locantore NW, Miller BE, et al. (2009) Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD. Eur Respir J 34: 93–102.

30. Shkapoers WA, Sin DD, Ghafor F, Bashir S, Bokhari SN (2009) Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease. Dis Markers 27: 287–294.

31. Winkler C, Atochica-Vasserman EN, Holz O, Beers MF, Eperenbeek VJ, et al. (2011) Comprehensive characterization of pulmonary and serum surfactant protein D in COPD. Respir Res 12: 29.

32. Jo CR, Liu W, Chen RC (2012) Serum surfactant protein D: biomarker of chronic obstructive pulmonary disease. Dis Markers 27: 281–287.

33. Ozuyrek BA, Ulasli SS, Bozhas SS, Bayraktar N, Akay S (2013) Value of serum and induced sputum surfactant protein-D in chronic obstructive pulmonary disease. Multidiscip Respir Med 8: 36.

34. El-Deek SE, Makhlouf HA, Saleem TM, Mandour MA, Mohamed NA (2013) Surfactant protein D, soluble intercellular adhesion molecule-1 and high-sensitivity C-reactive protein as biomarkers of chronic obstructive pulmonary disease. Med Princ Pract 22: 469–474.

35. Atkinson JJ, Layet BA, Suzuki Y, Toennis HM, Kelley DG, et al. (2011) The role of matrix metalloproteinase-9 in cigarette smoke-induced emphysema. Am J Respir Crit Care Med 183: 676–684.

36. Krane M, Griesse M (2003) Surfactant protein D in serum from patients with allergic bronchopulmonary aspergillosis. Eur Respir J 22: 592–595.

37. Olesen HV, Holmskov U, Schiott PO, Sorensen GL (2010) Serum-surfactant SP-D correlates inversely to lung function in cystic fibrosis. J Cyst Fibros 9: 257–262.

38. Hill J, Hedgpeth C, Man SF, Frohlich J, Connell JE, et al. (2011) Circulating surfactant protein-D and the risk of cardiovascular morbidity and mortality. Eur Heart J 32: 1918–1925.

39. Engels GE, Gu VJ, van Oeveren W, Rakhiosis G, Mariani MA, et al. (2013) The utility of lung epithelium specific biomarkers in cardiac surgery: a comparison of biomarker profiles in off- and on-pump coronary bypass surgery. J Cardiothorac Surg 8: 4.

40. Elhajj M, Charles J, Pedroza C, Liu X, Zhou X, et al. (2013) Can serum SP-D Levels in Blood as Measured by ELISA
67. Hoegh SV, Sorensen GL, Tornoe I, Lottenburger T, Ytting H, et al. (2010) Functional and biological characteristics of asthma in cleaning workers. Respir Med 104: 673–683.

56. Bejvl I, Weseslindtner L, Strassl R, Jaksch P, Kundi M, et al. (2013) Analysis of surfactant protein D serum levels as a biomarker of lung injury in respiratory syncytial virus bronchiolitis. Pediatr Pulmonol 48: 338–366.

55. Wu YP, Liu ZH, Wei R, Pan SD, Mao NY, et al. (2009) Elevated plasma levels of Clara cell protein 16 and surfactant protein-D in the early diagnosis and progression of lung cancer. J Occup Environ Med 51: 834–839.

54. Christensen AF, Sorensen GL, Horslev-Petersen K, Holmskov U, Lindegaard JK (2012) Blood biomarkers and measures of pulmonary function–a study in a rural Danish cohort. Thorax 67: 596–601.

53. Haddan N, Samara S, Dumont X, Taleb A, HAufroid V, et al. (2009) Lung epithelial injury biomarkers in workers exposed to sulphur dioxide in a non-ferrous smelter. Biomarkers 14: 292–298.

52. Higashi A, Higashi N, Tsuburai T, Takeuchi Y, Taniguchi M, et al. (2005) Serum KL-6 and surfactant proteins A and D in pediatric interstitial lung disease in patients with scleroderma. J Rheumatol 32: 1023–1032.

51. Higashi A, Higashi N, Tsuburai T, Takeuchi Y, Taniguchi M, et al. (2005) Serum KL-6 and surfactant protein D in children with 2009 pandemic H1N1 influenza infection. Pediatr Pulmonol 46: 18–22.

50. Hermans C, Bernard A (1999) Lung epithelium-specific proteins: characteristics and functional significance of surfactant protein D as a serum marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. Arthritis Rheum 44: 1363–1369.

49. Janssen R, Sato H, Grutters JC, Bernard A, van Velzen-Blad H, et al. (2003) Surfactant protein D serum levels in patients following acute paracetamol intoxication. Clin Toxicol (Phila) 41: 1268–1272.

48. Janssens RJ, Bellamy SL, Localio AR, Wickersham N, Diamond JM, et al. (2012) A panel of lung injury biomarkers enhances the definition of primary graft dysfunction (PGD) after lung transplantation. J Heart Lung Transplant 31: 942–949.

47. Hoffmann HJ, Iversen M, Brandslund I, Sigsgaard T, Omland O, et al. (2003) Pulmonary surfactant protein D and measurement of its blood levels in drowning victims. Forensic Sci Int 109: 51–63.

46. Hong Y, Va Asano Y, Kubo M, Yamanaka K, Fujimi M, et al. (2002) Clinical significance of serum surfactant protein-D (SP-D) in patients with polymyxin/ dermatomyositis: correlation with interstitial lung disease. Rheumatol (Oxford) 41: 1268–1272.

45. Haddan N, Samara S, Dumont X, Taleb A, HAufroid V, et al. (2009) Lung epithelial injury biomarkers in workers exposed to sulphur dioxide in a non-ferrous smelter. Biomarkers 14: 292–298.

44. Asano Y, Iba H, Yamane K, Yasawa N, Kubo M, et al. (2001) Clinical significance of surfactant protein D as a serum marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. Arthritis Rheum 44: 1363–1369.

43. Al-Salmi QA, Walter JN, Colasurdo GN, Sockrider MM, Smith EO, et al. (2009) Involvement of eosinoclasts and surfactant protein D in extrinsic allergic alveolitis. Eur J Resp Dis 169: 1069–1073.

42. Vincaya D, Mirabelli MC, Orruolo R, Anto JM, Barreiro E, et al. (2013) Biological characteristics of asthma in cleaning workers. Respir Med 107: 673–683.

41. Janssen R, Sato H, Grutters JC, Bernard A, van Velzen-Blad H, et al. (2003) Study of Clara cell 16, KL-6, and surfactant protein-D in serum as disease markers in pulmonary sarcoidosis. Chest 124: 2119–2125.

40. Koopmans JM, van der Zee JS, Krop EF, Lepuaha CE, Jansen HM, et al. (2004) Surfactant protein D is elevated in allergic patients. Clin Exp Allergy 34: 1827–1833.

39. Higashi A, Higashi N, Tsuburai T, Takeuchi Y, Taniguchi M, et al. (2005) Evidence that serum levels of surfactant protein-D (SP-D) are genetically influenced. Immunogenetics 57: 1–7.

38. Leth-Larsen R, Garred P, Holetz B, Schlichter S, Hartshorn K, et al. (2009) A common polymorphism in the SFTPD gene influences assembly, function, and concentration of surfactant protein-D. J Immunol 184: 1532–1538.

37. Zhao XM, Wu YF, Wei R, Cai HX, Tornoe I, et al. (2007) Plasma surfactant protein-D levels and the relation to body mass index in a Chinese population. Scand J Immunol 66: 71–76.

36. Hoffmann HJ, Iversen M, Brandslund I, Sigsgaard T, Omland O, et al. (2003) Plasma KL-6 levels of young farmers correlate with respirable dust exposure levels during normal work in swine confinement buildings. Ann Agric Environ Med 10: 53–60.

35. Van Miert E, Sardella A, Nickmilder M, Bernard A (2012) Respiratory effects associated with wood fuel use: a cross-sectional biomarker study among adolescents. Pediatr Pulmonol 47: 358–366.

34. Wang SX, Liu P, Wei MT, Chen L, Gao Y, et al. (2007) Roles of serum clara cell protein 16 and surfactant protein-D in the early diagnosis and progression of lung cancer. J Occup Environ Med 49: 834–839.

33. Kobayashi H, Kano H, Motoyoshi K (2008) Serum surfactant protein-A, but not surfactant protein-D or KL-6, can predict preclinical lung damage induced by smoking. Biomarkers 13: 385–392.

32. Van Miert E, Sardella A, Bernard A (2011) Biomarkers of early respiratory effects in smoking adolescents. Eur Respir J 38: 1287–1293.

31. Shaels MS, Chaturvedi AK, Katki HA, Gochouc BR, Caporaso NE, et al. (2011) Circulating markers of interstitial lung disease and subsequent risk of lung cancer. Cancer Epidemiol Biomarkers Prev 20: 259–267.

30. Aul R, Armstrong J, Dovox A, Loanas D, Hayes B, et al. (2012) Inhaled LPS challenges in smokers: a study of pulmonary and systemic effects. Br J Clin Pharmacol 74: 1023–1032.

29. Gramm F, Royniner G, Recknor L, Wallenius J, Diehl S, et al. (2002) The effects of GH and hormone replacement therapy on serum concentrations of mamman-binding lectin, surfactant protein D and vitamin D binding protein in Turner syndrome. Eur J Endocrinol 150: 355–362.

28. Ilg HV, Hong JR, Park JH, Seo YS, Yang J0, et al. (2005) Plasma surfactant D in patients following acute paracetamol intoxication. Clin Toxicol (Phila) 43: 463–467.

27. Shah R, Bellamy SL, Localio AR, Wickersham N, Diamond JM, et al. (2012) A panel of lung injury biomarkers enhances the definition of primary graft dysfunction (PGD) after lung transplantation. J Heart Lung Transplant 31: 942–949.

26. Duca I, Grzybowska K, Jedrzejowska-Syczpula H, Lewin-Kowalsk J (2012) The siting position during neurosurgical procedures does not influence serum biomarkers of pulmonary parenchymal injury. BMC Surg 12: 24.

25. Kamada S, Seo Y, Takahama K (2000) A sandwich enzyme immunoassay for pulmonary surfactant protein D and measurement of its blood levels in drowning victims. Forensic Sci Int 109: 51–63.

24. Rin H, Va Asano Y, Kubo M, Yamanaka K, Fujimi M, et al. (2002) Clinical significance of serum surfactant protein-D (SP-D) in patients with polymyxin/dermatomyositis: correlation with interstitial lung disease. Rheumatol (Oxford) 41: 1268–1272.

23. Nybo M, Andersen K, Sorensen GL, Loek A, Krakh-Sorensen P, et al. (2007) Serum surfactant protein D is correlated to development of dementia and augmented mortality. Clin Immunol 123: 333–337.

22. Haddan N, Samara S, Dumont X, Taleb A, HAufroid V, et al. (2009) Lung surfactant protein D levels is decreased in systemic lupus erythematosus. J Rheumatol 36: 2449–2453.

21. Aihara K, Oga T, Harada Y, Chihara Y, Handa T, et al. (2011) Comparison of biomarkers of subclinical lung injury in obstructive sleep apnea. Respir Med 105: 939–945.