Circulating surfactant protein-D and the risk of cardiovascular morbidity and mortality

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Aims
Surfactant protein-D (SP-D) is a lung-specific protein that is detectable in human plasma. We determined the relationship of circulating SP-D to cardiovascular disease (CVD) and total mortality in subjects with and without CVD.

Methods and results
Plasma SP-D levels were measured in 806 patients who underwent coronary angiography to assess its predictive value for cardiovascular mortality. Serum SP-D levels were also measured in a replication cohort to assess its relationship with CVD events in 4468 ex- and current smokers without a known history of coronary artery disease (CAD). Patients who died during follow-up had significantly higher plasma SP-D levels than those who survived (median 85.4 vs. 64.8 ng/mL; \(P < 0.0001\)). Those in the highest quintile of SP-D had 4.4-fold higher risk of CVD mortality than those in the lowest quintile (\(P < 0.0001\)) independent of age, sex, and plasma lipid levels. In a group of current and ex-smokers without a known history of CAD, serum SP-D levels were elevated in those who died or were hospitalized for CVD compared with those who did not (median 99.8 vs. 90.6 ng/mL; \(P = 0.0001\)).

Conclusion
Circulating SP-D is a good predictor of cardiovascular morbidity and mortality and adds prognostic information to well-established risk factors such as age, sex, and plasma lipids and is a promising biomarker to link lung inflammation/injury to CVD.

Keywords
Heart disease • Lung inflammation • Biomarkers • Surfactant • Cardiovascular mortality

Introduction
Despite two decades of significant progress in the management of patients with cardiovascular diseases (CVDs), heart and blood vessel diseases remain the leading cause of mortality in the western world.1 Although it has been known for years that cigarette smoking and impaired lung function are powerful, independent risk factors for CVDs, with population attributable risks that are similar to those of hypertension and hyperlipidaemia, they have been largely ignored as possible targets of drug or biomarker discoveries for CVDs.2 Recent studies indicate that surfactant protein-D (SP-D), a hydrophilic multimeric protein that is synthesized predominantly by type 2 pneumocytes in the lungs, is a promising blood biomarker of lung inflammation and injury related to cigarette smoking and chronic obstructive pulmonary disease.3 Although circulating SP-D levels are measurable in human plasma, its overall systemic expression is low. However, with lung injury and inflammation, lungs become more permeable, allowing SP-D to translocate readily into the systemic circulation.4 In the lungs, SP-D has major anti-microbial and anti-inflammatory effects;5 however, in the systemic circulation, it may disturb lipid metabolism and enhance the risk for atherosclerosis.6 However, the relationship of circulating SP-D to clinical outcomes such as cardiovascular morbidity and mortality has not been elucidated. In the present study, we determined whether or not circulating SP-D is related to cardiovascular morbidity and mortality in two independent cohorts: first, a large cohort of patients who underwent coronary angiography for suspected coronary artery
disease (CAD) and second, a cohort of smokers with mild airflow limitation and without a known history of CVD.

Methods

The first cohort

The first set of subjects for the study were derived from the Vancouver Coronary Angiography Cohort, which has been described previously in detail. In brief, the cohort was composed of men and women, who were referred for selective coronary angiography between 1992 and 1995 at two major teaching hospitals in Vancouver, Canada. Indications for angiography included stable angina, previous myocardial infarction, aortic valve disease, and mitral valve regurgitation. Patients with unstable angina or myocardial infarction within the preceding 2 months were excluded. Lesions visualized in major epicardial vessels were assessed semi-quantitatively for per cent stenosis, rounded to the nearest 10%. The presence of CAD was defined by the presence of any lesion causing ≥20% stenosis, and severe CAD was defined by the presence of any lesion causing ≥50% stenosis. A detailed questionnaire which included patient demographics, a clinical history of smoking (past, current, or never), hypertension, diabetes mellitus, and family history of premature CAD defined as a first-degree relative affected before age 45 years for men and before 55 years for women were obtained by self-report. Height and weight were measured from which body mass index (BMI) was calculated using a standard equation. Waist circumference was measured mid-way between the iliac crest and the bottom of the ribcage. Medication use was recorded from the patient’s chart.

Measurements

Before angiography, with the patient seated, a fasting blood sample was taken from the antecubital fossa using standard techniques. Blood samples were centrifuged immediately and aliquoted into smaller vials and frozen in −70°C until use. Plasma lipid levels were determined at the time of the initial blood collection, while plasma C-reactive protein and interleukin-6 (IL-6) were measured in 2002 using a high-sensitivity assay as previously reported. Plasma SP-D was measured in 2010 in duplicate using a commercially available solid-phase sandwich enzyme-linked immunosorbent assay (ELISA; BioVendor Laboratory Medicine, Modrice, Czech Republic) by personnel, who were blinded to the clinical characteristics of the subjects. A detailed questionnaire which included patient demographics, a clinical history of smoking (past, current, or never), hypertension, diabetes mellitus, and family history of premature CAD defined as a first-degree relative affected before age 45 years for men and before 55 years for women were obtained by self-report. Height and weight were measured from which body mass index (BMI) was calculated using a standard equation. Waist circumference was measured mid-way between the iliac crest and the bottom of the ribcage. Medication use was recorded from the patient’s chart.

Follow-up

Subjects were followed passively until November 2007. The vital statistics of the subjects were ascertained using the British Columbia Vital Statistics Agency mortality database. The primary outcome in the Vancouver Angiography Cohort was CVD mortality, which was determined using the World Health Organization International Classification of Disease-10th revision mortality codes I20–I25 and I60–I69.

The replication cohort

The details of Lung Health Study (LHS) have been previously published. Briefly, LHS recruited 5887 cigarette-smoking individuals between the ages of 35 and 60 years who had mild-to-moderate airflow obstruction on spirometry defined by a forced expiratory volume in one second (FEV1) to forced vital capacity ratio of <0.70 and FEV1 between 55 and 90% of predicted normal values. Individuals who had a history of cancer (except carcinoma in situ or basal cell carcinoma of the skin), myocardial infarction (in the past 2 years), angina, heart failure, stroke (in the past 2 years), renal failure, insulin-requiring diabetes mellitus, cirrhosis or other serious liver diseases, pulmonary embolism, disorders of the central nervous system, narrow-angle glaucoma, or any other major diseases that could have compromised follow-up were excluded.

Measurements

Lung Health Study participants were followed yearly. At the fifth annual visit, venipuncture was carried out on the participants. There were 5413 subjects who were alive and eligible for venipuncture at this visit. Of these, 4754 (88% of eligible subjects) had sufficient volume of serum for the protein measurement. Using one aliquot of serum samples, we determined SP-D levels as we did for the angiography cohort using the same ELISA kits. The laboratory personnel were blinded to the clinical characteristics of the study patients.

Follow-up

During the subsequent follow-up phase, the participants’ vital status was captured every 6 months. An independent mortality and morbidity committee reviewed death certificates, autopsy reports, relevant hospital records, and summaries of interviews with attending physicians, or eyewitnesses and assigned the causes of death for all participants who died during the study. These data were supplemented by linkages with a National Death Index, which provided the date and cause of death for all US study participants through the end of 2001. Vital status was successfully determined for 98.3% of the participants. Because the blood samples were taken at Year 5, individuals who died during the first 5 years of LHS follow-up or experienced a CVD event prior to blood sampling were excluded from the present analysis.

Statistical analysis

Circulating SP-D concentrations were divided in quintiles from the lowest to the highest levels. We compared the risk of CVD mortality across the quintiles over the follow-up period using a Kaplan–Meier (K–M) method for the univariate analysis and a Cox proportional hazards model for the multivariate analysis. The K–M survival curves across the SP-D quintiles were compared using a log-rank test. As the upper limit of normal for SP-D in the non-smoking individuals is 175.5 ng/mL, we also performed the analysis using this cut-off. In the multivariate model, we included the following covariates: age, sex, BMI, systolic blood pressure, current smoking status (ex vs. current smoker vs. non-smoker), plasma low-density lipoprotein cholesterol (LDLc), plasma high-density lipoprotein cholesterol (HDLc), total cholesterol, plasma C-reactive protein, history of diabetes mellitus and hypertension, presence of severe CAD on angiography (i.e. ≥50% stenotic lesion), presence of triple vessel disease on angiography, and use of aspirin, angiotensin-converting enzyme inhibitor, anti-lipid drugs, and beta-blockers. To avoid collinearity and to make the model efficient and parsimonious, we used the selection strategy of Shtatland et al., which allows covariates to be chosen based on optimal Akaiake Information Criterion (AIC) values on a full set of models. The most optimal AIC value was obtained by the inclusion of age, plasma IL-6 levels, serum triglycerides, current smoking status, use of angiotensin-converting enzyme, presence of triple vessel disease on angiography, and presence of diabetes as covariates. We determined the receiver-operating characteristic (ROC) curve and the c statistics to determine the performance of SP-D in discriminating patients who did and did not die from CVDs and used the method of Pencina et al. to re-classify patients for...
the risk of CVD mortality based on the inclusion of plasma SP-D to the traditional risk factors. We repeated these analyses using all-cause mortality as a secondary endpoint. We used a similar approach for the LHS cohort except in the adjusted model, we included as covariates: age, sex, race, BMI, and pack-years of smoking. The LHS cohort was used as a replication cohort. The primary endpoint for this cohort was cardiovascular hospitalization or mortality, whichever came first. We used a similar survival analysis for this cohort. Continuous variables were presented as mean ± SD [or median ± median absolute deviation (MAD) for non-normally distributed variable] and dichotomous variables as number (%) of total. The fit of the model was tested using the Hosmer–Lemeshow Goodness of Fit Test. All analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). P-values < 0.05 were considered to indicate statistical significance.

**Results**

**Vancouver Coronary Angiography Cohort**

Of the 806 patients in whom plasma levels of SP-D were determined, 240 (30% of patients) died during a mean follow-up period of 12.82 ± 0.43 years (median ± MAD). There were 107 CVD deaths accounting for 45% of the total mortality. Eight per cent of the patients had CAD (verified on angiography), 71% had severe CAD, and 29% had angiographic evidence of triple vessel disease. The average age of the patients at the time of blood sampling was 61.2 ± 10.9 years; 584 (75%) were men, 548 (71%) were white, and 208 (27%) patients were lifetime non-smokers. Only 3% of the current smokers did not have an atherosclerotic lesion on angiography. The average BMI was 28.0 ± 4.8 kg/m² and the waist circumference was 91.9 ± 25.9 cm. Diabetes mellitus was reported in 138 (18%) of the patients. The average plasma total cholesterol was 5.10 ± 1.10 mmol/L. LDL cholesterol was 3.50 ± 1.05 mmol/L, HDL cholesterol was 0.93 ± 0.26 mmol/L, triglycerides was 1.85 ± 1.32 mmol/L, and apolipoprotein B was 0.96 ± 0.24 mmol/L. The average left ventricular ejection fraction on two-dimensional echocardiography was 63.1 ± 13.1%. The median (± MAD) plasma C-reactive protein was 1.70 ± 1.81 mg/L, while that for IL-6 was 2.27 ± 1.49 pg/mL and SP-D was 72.3 ± 40.6 ng/mL.

**Comparisons across quintiles of surfactant protein-D**

Demographic and clinical profile of patients across the SP-D quintiles were similar except for age, which increased as SP-D levels increased, and BMI, which decreased as SP-D levels increased (Table 1). Patients who died during follow-up had significantly higher plasma SP-D concentrations than those who survived (median ± MAD, 85.4 ± 48.3 vs. 64.8 ± 35.6 ng/mL; P < 0.0001). The increased cardiovascular disease mortality across the SP-D quintiles is depicted in Figure 1. In total, 11 patients (6.8%) in Quintile 1, 12 (7.5%) in Quintile 2, 20 (12.4%) in Quintile 3, 31 (19.3%) in Quintile 4, and 33 (20.5%) died of CVD causes (P for trend < 0.0001). Total mortality also increased along the SP-D gradient (see Supplementary material online, Figure S1). There were 31 deaths (19.3% of patients) in Quintile 1, 35 deaths (21.7%) in Quintile 2, 44 deaths (27.2%) in Quintile 3, 56 deaths (34.8%) in Quintile 4, and 74 (46.0%) deaths in Quintile 5 of SP-D during follow-up (P for trend < 0.0001; Table 2). Compared with Quintile 1, the relative risk (RR) of total mortality was 3.1-fold higher in Quintile 5, 2.0-fold higher in Quintile 4, 1.62-fold higher in Quintile 3, and 1.29-fold higher in Quintile 2 (P for trend < 0.0001) adjusted for covariates (Table 2). Nearly 45% of the total deaths were cardiovascular mortality (n = 107). Quintile 1 had 11 CVD deaths (6.8%), Quintile 2 had 12 (7.5%) CVD deaths, Quintile 3 had 20 (12.4%) CVD deaths, Quintile 4 had 31 (19.3%) CVD deaths, and Quintile 5 had 33 CVD deaths (20.5%). Compared with Quintile 1, Quintile 5 had 4.4-fold increase in the risk for CVD mortality, Quintile 4 had 3.8-fold increase in the risk, Quintile 3 had 2.3-fold increase in the risk, and Quintile 2 had 1.6-fold increase in the risk of CVD mortality (Table 2). Total mortality had a similar trend as that for CVD mortality (Table 2 and see Supplementary material online, Figure S1). The upper limit of normal for SP-D in the non-smoking individuals is 175.5 ng/mL. The adjusted hazard ratio of CVD mortality for individuals with SP-D greater than or equal to this cut-off was 4.11 (95% confidence interval (CI): 2.49–6.78) and that for total mortality was 3.10 (95% CI: 2.15–4.48) compared with those with SP-D levels below this level (Figure 2). The fit of the model was acceptable (P = 0.373 for mortality and P = 0.794 for CVD mortality).

**The value of surfactant protein-D as a predictor of cardiovascular disease and total mortality in the Vancouver Angiography Cohort**

The variables age, sex, smoking status, plasma IL-6 levels, systolic blood pressure, plasma LDL cholesterol levels, and presence of triple vessel disease on cardiac angiography and use of angiotensin-converting enzyme inhibitors were collectively associated with a ROC curve area of 0.760 for CVD mortality. Individual ROC curve areas are summarized in Table 3. The addition of plasma SP-D values increased the ROC curve area to 0.777 (P = 0.0005) and to an integrated discrimination improvement of 0.0233 ± 0.0053 (SE, P < 0.0001). The net reclassification improvement (NRI) for CVD mortality was calculated from patient reclassification rates conferred by SP-D. Surfactant protein-D improved risk prediction across all levels of risk (see Supplementary material online, Table S1), with an NRI of 12.79% ± 6.47% (P = 0.0479). The addition of SP-D values to the prediction model also enhanced the ROC curve area for total mortality (from 0.746 to 0.760; P < 0.0001). Surfactant protein-D improved risk prediction across all levels of risk (see Supplementary material online, Table S2) with an NRI of 11.07% ± 5.57% (P = 0.0468).

**Lung Health Study cohort**

There were 4754 subjects in whom serum levels of SP-D were determined. Of these subjects, 327 (6.9% of the subjects) died during a median follow-up period of 13.83 ± 0.83 years (median ± MAD). There were 87 CVD deaths accounting for 26.6% of the total mortality. An additional 727 subjects experienced CVD hospitalization. Thus, in total, 814 (17.1% of the
subjects) experienced a CVD event defined as CVD hospitalization or mortality. The average age of the patients at the time of blood sampling was 53.5 ± 6.8 years; 2998 (63.1%) were men, 4557 (96.3%) were white, and 2575 (54.2%) subjects were continuous smokers. The average BMI was 25.6 ± 3.9 kg/m² and FEV₁ was 75.0 ± 12.3% of predicted. The median ± MAD serum C-reactive protein was 1.40 ± 1.41 mg/L, while that for IL-6 was 2.62 ± 1.56 pg/mL and SP-D was 92.6 ± 45.3 ng/mL.

Comparisons across quintiles of surfactant protein-D for Lung Health Study

Demographic and clinical profiles of patients across the SP-D quintiles are summarized in Supplementary material online, Table S3. Both total and CVD mortality increased across the SP-D quintiles (see Supplementary material online, Figure S2). There were 51 deaths (5.4% of patients) in Quintile 1, 69 deaths (7.2%) in Quintile 2, 57 deaths (6.0%) in Quintile 3, 70 deaths (7.4%) in Quintile 4, and 80 (8.4%) deaths in Quintile 5 of SP-D during follow-up (P for trend = 0.0171). There were 175 CVD events, which were defined as cardiovascular hospitalization or mortality, in Quintile 1 (18.4% of patients), 190 (or 20.0%) in Quintile 2, 201 (21.1%) in Quintile 3, 213 (22.4%) in Quintile 4, and 241 (25.3%) in Quintile 5 (P for trend = 0.0001; see Supplementary material online, Table S3). The RR for CVD events increased along the serum gradient of SP-D with or without adjustments for covariates (see Supplementary material online, Table S4). Individuals who experienced CVD events had higher SP-D levels than those who did not (median ± MAD: 98.0 ± 48.0 vs. 90.6 ± 44.6 ng/mL; P = 0.030).

Discussion

We found that circulating SP-D, a hydrophilic molecule synthesized predominantly by type 2 alveolar cells in the lungs, was associated with CVD and total mortality in patients with documented CAD, independent of other well-established risk factors such as age, sex, cigarette smoking, plasma cholesterol, and plasma IL-6

Table 1  Demographic and clinical characteristics of the Vancouver Angiography Cohort across quintiles of plasma surfactant protein-D

<table>
<thead>
<tr>
<th>Quint 1 (n = 161)</th>
<th>Quint 2 (n = 161)</th>
<th>Quint 3 (n = 162)</th>
<th>Quint 4 (n = 161)</th>
<th>Quint 5 (n = 161)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-D, ng/mL</td>
<td>≤43.0</td>
<td>43.1–61.4</td>
<td>61.4–81.5</td>
<td>81.5–114.5</td>
<td>114.6–531.2</td>
</tr>
<tr>
<td>Age, years</td>
<td>59.1 ± 11.9</td>
<td>60.1 ± 10.7</td>
<td>60.4 ± 11.5</td>
<td>62.6 ± 9.9</td>
<td>63.6 ± 9.8</td>
</tr>
<tr>
<td>Men, %</td>
<td>76.1</td>
<td>72.2</td>
<td>74.3</td>
<td>75.8</td>
<td>79.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.9 ± 5.8</td>
<td>28.0 ± 4.4</td>
<td>27.9 ± 5.0</td>
<td>27.9 ± 4.7</td>
<td>27.4 ± 4.0</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>90.8 ± 29.4</td>
<td>92.9 ± 21.6</td>
<td>91.2 ± 28.5</td>
<td>93.9 ± 23.0</td>
<td>90.9 ± 26.2</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>7.8</td>
<td>13.2</td>
<td>20.8</td>
<td>21.4</td>
<td>15.4</td>
</tr>
<tr>
<td>Ex-smokers, %</td>
<td>57.1</td>
<td>57.6</td>
<td>49.0</td>
<td>54.5</td>
<td>66.5</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>76.1 ± 17.6</td>
<td>73.1 ± 22.9</td>
<td>75.5 ± 18.6</td>
<td>74.9 ± 18.5</td>
<td>75.0 ± 17.1</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>130.3 ± 31.3</td>
<td>124.38.3</td>
<td>128.7 ± 33.9</td>
<td>129.1 ± 36.6</td>
<td>131.6 ± 32.2</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>42.7</td>
<td>37.0</td>
<td>42.5</td>
<td>37.7</td>
<td>42.7</td>
</tr>
<tr>
<td>Diabetes mellitus,%</td>
<td>20.0</td>
<td>16.1</td>
<td>18.4</td>
<td>17.8</td>
<td>16.8</td>
</tr>
<tr>
<td>Presence of vessel with &gt;50% stenosis, %</td>
<td>69.3</td>
<td>63.9</td>
<td>70.4</td>
<td>77.4</td>
<td>72.5</td>
</tr>
<tr>
<td>Presence of triple vessel disease, %</td>
<td>24.8</td>
<td>22.4</td>
<td>33.6</td>
<td>31.0</td>
<td>33.6</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>70.3</td>
<td>72.9</td>
<td>59.2</td>
<td>75.6</td>
<td>65.2</td>
</tr>
<tr>
<td>ACE-inhibitors, %</td>
<td>17.4</td>
<td>13.5</td>
<td>22.4</td>
<td>19.1</td>
<td>23.9</td>
</tr>
<tr>
<td>Beta-blockers, %</td>
<td>41.9</td>
<td>56.1</td>
<td>44.1</td>
<td>57.3</td>
<td>41.9</td>
</tr>
<tr>
<td>Anti-lipids, %</td>
<td>16.8</td>
<td>16.1</td>
<td>20.4</td>
<td>16.6</td>
<td>14.2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.89 ± 0.89</td>
<td>5.21 ± 1.11</td>
<td>5.14 ± 1.21</td>
<td>5.23 ± 0.96</td>
<td>5.02 ± 1.25</td>
</tr>
<tr>
<td>LDLc, mmol/L</td>
<td>3.34 ± 0.85</td>
<td>3.55 ± 1.12</td>
<td>3.52 ± 1.15</td>
<td>3.58 ± 0.98</td>
<td>3.52 ± 1.10</td>
</tr>
<tr>
<td>Oxidized LDLc, mmol/L</td>
<td>72.7 ± 26.2</td>
<td>78.8 ± 23.5</td>
<td>76.1 ± 23.6</td>
<td>78.6 ± 24.0</td>
<td>75.6 ± 20.1</td>
</tr>
<tr>
<td>HDLc, mmol/L</td>
<td>0.93 ± 0.25</td>
<td>0.94 ± 0.26</td>
<td>0.94 ± 0.24</td>
<td>0.92 ± 0.27</td>
<td>0.92 ± 0.27</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.52 ± 0.91</td>
<td>1.63 ± 1.00</td>
<td>1.66 ± 0.95</td>
<td>1.65 ± 1.01</td>
<td>1.49 ± 0.89</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.29 ± 3.33</td>
<td>1.95 ± 2.47</td>
<td>1.56 ± 2.14</td>
<td>1.94 ± 2.35</td>
<td>2.36 ± 2.56</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.19 ± 1.79</td>
<td>2.17 ± 2.11</td>
<td>1.99 ± 1.69</td>
<td>2.21 ± 1.60</td>
<td>2.75 ± 1.77</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>67.4 ± 9.6</td>
<td>67.7 ± 11.0</td>
<td>61.6 ± 13.5</td>
<td>62.2 ± 13.3</td>
<td>58.8 ± 15.7</td>
</tr>
</tbody>
</table>

ACE: angiotensin-converting enzyme; BMI: body mass index; CRP: C-reactive protein; HDLc: high-density lipoprotein cholesterol; IL-6: interleukin-6; LDLc: low-density lipoprotein cholesterol; LVEF: left ventricular ejection fraction; Quint: quintile groups; SP-D: surfactant protein-D.

*Median ± median absolute deviation.
Surfactant protein-D was more discriminative of CVD and total mortality than any of these factors except for age. These data are consistent with those of Lomas et al. and Nybo et al. Both studies demonstrated a significant association of serum SP-D with total mortality. However, neither of these studies had sufficient power to assess cardiovascular endpoints. We extend these data by demonstrating a strong and consistent relationship of SP-D to CVD hospitalization and mortality in older patients with established CAD and in younger individuals without a history of CAD.
It has been long known that chronic lung inflammation is associated with increased risk of cardiovascular and total mortality; however, aside from lung function measurements, there are no universally accepted biomarkers that could predict these events. Recent studies indicate that serum or plasma SP-D is a promising biomarker of lung inflammation and injury.3,15–17 In general, circulating SP-D levels are nearly 40% higher in active smokers compared with lifetime non-smokers3 and rise further in subjects with impaired lung function.7 Paradoxically, the lung expression of SP-D in smokers and those with impaired lung function decreases relative to lifetime non-smokers,18 suggesting that circulating SP-D levels rise in response to increased translocation of SP-D from the lungs into the systemic circulation. By adjusting for cigarette smoking, we could have underestimated the ‘true’ relationship of blood SP-D to cardiovascular morbidity and mortality because it is possible that SP-D may be in the causal

![Figure 2](https://academic.oup.com/eurheartj/article-abstract/32/15/1918/566633)

**Figure 2** The risk of total cardiovascular mortality between individuals with plasma surfactant protein-D above or below the upper limit of normal (surfactant protein-D level of 175.5 ng/mL) values in the non-smoking population. Log-rank comparison $P < 0.0001$. In this analysis, surfactant protein-D value of 175.5 ng/mL was used to dichotomize the cohort. This value represents the 95 percentile limit of surfactant protein-D in the non-smoking adult population.3 Patients with plasma surfactant protein-D levels above this threshold had a significantly higher risk of cardiovascular mortality than those below this level.

### Table 3

<table>
<thead>
<tr>
<th>Variables</th>
<th>CVD mortality</th>
<th>Total mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-statistics</td>
<td>$P$-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.678 ± 0.029</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SP-D</td>
<td>0.651 ± 0.028</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.630 ± 0.029</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of triple vessel disease</td>
<td>0.618 ± 0.031</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Use of ACE-inhibitors</td>
<td>0.605 ± 0.031</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All other variables were not significantly related to CVD or total mortality.

ACE, angiotensin-converting enzyme; IL-6, interleukin-6; SP-D, surfactant protein-D.
pathway. Furthermore, elevated serum SP-D levels have been associated with increased risk of chronic obstructive pulmonary disease exacerbations and dementia.14

The precise role of SP-D has not been fully elucidated. However, in the lungs, SP-D plays an active role in bacterial clearance and in regulating airway inflammation.19 Surfactant protein-D binds to specific lung antigens and modulates the host inflammatory reaction.20,21 Surfactant protein-D also facilitates efferocytosis by binding to dying and dead cells and facilitating their removal from the lungs, which is a critical step in resolving inflammation.22,23 Additionally, SP-D attenuates oxidant stress in the lungs by inhibiting the formation of lipid radicals and acting as free radical chain terminators, whereas SP-D deficiency enhances oxidative lung injury.24 Mice deficient in SP-D develop emphysema-like lung disease exacerbations3 and dementia.14

In summary, we found that circulating SP-D levels are strongly predictive of future risk of cardiovascular mortality, independent of well-established risk factors. These data implicate lung inflammation in the pathogenesis of heart and blood vessel disease and raise the possibility of using this protein as a biomarker to risk-stratify CVD patients above and beyond traditional risk factors such as serum cholesterol and C-reactive protein. Since serum SP-D rises with lung injury (as with chronic obstructive pulmonary disease),3 raised systemic levels of SP-D in CVD patients should alert the practicing clinicians to consider chronic airway diseases, which can be objectively verified using simple spirometry.28

### Supplementary material

Supplementary material is available at European Heart Journal online.

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### Conflict of interest

none declared.

### References