Plasma Surfactant Protein-B
A Novel Biomarker in Chronic Heart Failure

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Background—In chronic heart failure (CHF), elevated pulmonary microvascular pressure (P mv ) results in pulmonary edema. Because elevated P mv may alter the integrity of the alveolocapillary barrier, allowing leakage of surfactant protein-B (SP-B) from the alveoli into the circulation, we aimed to determine plasma levels of SP-B in CHF and their relation to clinical status.

Methods and Results—Fifty-three outpatients with CHF had plasma SP-B and N-terminal proBNP (NT-proBNP) assayed, in addition to a formalized clinical assessment at each clinic review over a period of 18 months. The control group comprised 19 normal volunteers. Plasma SP-B was elevated in CHF (P<0.001), and levels increased with New York Heart Association classification (P<0.001). SP-B correlated with objective clinical status parameters and NT-proBNP. During follow-up, major cardiovascular events occurred in patients with higher plasma SP-B (P<0.01) and NT-proBNP (P<0.05). Furthermore, on conditional logistic regression analysis, only SP-B was independently associated with CHF hospitalization (P=0.005). The 53 patients underwent a total of 210 outpatient visits. When the diuretic dosage was increased on clinical grounds, SP-B had increased 39% (P<0.001) and NT-proBNP had increased 32% (P<0.001). Conversely, at the next visit, SP-B fell 12% (P<0.001), whereas NT-proBNP fell 39% (P<0.001).

Conclusions—Plasma SP-B is increased in CHF, and levels are related to clinical severity. Furthermore, within individual patients, SP-B levels vary with dynamic clinical status and NT-proBNP levels. Because plasma SP-B is independently associated with CHF hospitalization, it may, by virtue of its differing release mechanism to NT-proBNP, be a clinically useful biomarker of the pulmonary consequences of raised P mv . (Circulation. 2004;110:1091-1096.)

Key Words: edema ■ heart failure ■ lung ■ natriuretic peptides ■ proteins
increased microvascular protein permeability caused by “pore stretching” has been demonstrated.13 We have demonstrated increased leakage of SP-B into the plasma in acute cardiogenic pulmonary edema12 and have recently demonstrated increased alveolocapillary barrier protein permeability in a rat model of CHF,13 thereby providing a biological basis for increased SP-B leakage into the circulation in clinical CHF.

We hypothesized that plasma SP-B is increased in CHF in a manner reflecting disease severity. Our subsidiary hypotheses were (1) that by providing information on the pulmonary consequences of raised Pmv, SP-B may better predict future CHF hospitalization than plasma NT-proBNP and (2) that within individual patients, plasma NT-proBNP and SP-B levels will vary over time, reflecting clinical status through episodes of decompensated CHF.

Methods

The Flinders Medical Centre Ethics Committee approved this study, and all study subjects and control subjects gave written informed consent to participate.

Patients and Procedures

Fifty-three patients with CHF (18 female) from the Flinders Medical Centre Heart Failure Clinic were assessed longitudinally at each clinic visit over an 18-month period. The only exclusion criteria to study participation were primary lung disease (excluding chronic obstructive pulmonary disease)3 and inability to provide consent.

Assessment consisted of the following:

1. Dyspnea score (DS)4;
2. LV failure score (LVFS): objective signs and specific symptoms of LV failure, with scoring based on the Framingham criteria for diagnosing decompensated heart failure15 (scoring system: chest crepitations [basal=0.5, >1/3=1, >2/3=1.5], third heart sound [present=0.5, orthopnea [possible=0.5, definite=1], paroxysmal nocturnal dyspnea [<2 episodes/week=1, >3 episodes/week=2];
3. Six-minute walk test (6-MWT);
4. New York Heart Association (NYHA) functional class;
5. Body weight; and

The control group comprised 19 age-matched normal volunteers 72 years of age (59, 86).

Specimen Handling and Assays

Venous blood was collected in lithium-heparin tubes and centrifuged at 5000 rpm; the supernatant was frozen at −70°C for blinded batch analysis. NT-proBNP was measured with the use of a commercially available electrochemiluminescence sandwich immunoassay (proBNP, ELECSYS 2010, Roche Diagnostics). SP-B levels were measured with the use of competitive enzyme-linked immunosorbent assay.7

Statistical Analysis

Data were analyzed with the use of SPSS for Windows, release 10.0. Continuous variables were not normally distributed; hence, nonparametric data analysis was performed. All data are presented as median (25th, 75th percentiles), with statistical significance defined as P<0.05.

NT-proBNP and SP-B levels in patients with CHF were compared with levels in control subjects by means of the Mann-Whitney U test. Differences in measured parameters at the first clinic assessment according to NYHA class were tested by means of the Kruskal-Wallis test. Correlations were performed by means of Spearman’s test. A conditional logistic regression model was used to determine the predictive value of plasma NT-proBNP and SP-B for CHF hospitalization and death.

When the treating cardiologist elected to increase the loop-diuretic dosage at a clinic visit, the measured parameters were compared with those at the previous clinic visit and the subsequent clinic visit by means of the Wilcoxon signed ranks test.

Results

The CHF cohort patients were typical ambulatory heart failure clinic patients, with a median (25th, 75th percentiles) age of 67 years (55, 77 years), a median LV ejection fraction of 26% (20%, 30%), and predominately (70%) an ischemic cause of heart failure. Seventeen patients were initially in NYHA functional class II, 22 were in class III, and 14 were in class IV.

Measured Parameters Across NYHA Classification at Initial Visit

Increasing NYHA functional classification was associated with a poorer clinical state and prognosis (Table 1).

Plasma NT-proBNP was elevated in the patients with CHF (P<0.001). Indeed, plasma levels were elevated in the NYHA class II subgroup of patients compared with the control subjects. Furthermore, levels increased with NYHA classification (Figure 1A).

Plasma SP-B also was elevated in the patients with CHF (P<0.001). Again, plasma levels were elevated in the NYHA class II subgroup compared with the control subjects, and levels increased with NYHA classification (Figure 1B).

Correlations Between Measured Parameters

As expected, the DS, LVFS, and 6-MWT distances were related. Although NT-proBNP and SP-B were related, SP-B

<table>
<thead>
<tr>
<th>NYHA Functional Classification</th>
<th>Class II (n=17)</th>
<th>Class III (n=22)</th>
<th>Class IV (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>61 (52, 72)</td>
<td>68 (54, 73)</td>
<td>74 (67, 83)</td>
<td>0.019</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.27 (0.23, 0.35)</td>
<td>0.26 (0.2, 0.3)</td>
<td>0.26 (0.2, 0.3)</td>
<td>0.61</td>
</tr>
<tr>
<td>DS</td>
<td>8 (6, 10)</td>
<td>5 (4, 6)</td>
<td>4 (3, 4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVFS</td>
<td>0 (0, 0.25)</td>
<td>0.25 (0, 1.125)</td>
<td>1.5 (0.5, 4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-MWT, m</td>
<td>450 (400, 567)</td>
<td>366 (293, 394)</td>
<td>100 (50, 160)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CHF admission</td>
<td>2 (12%)</td>
<td>6 (27%)</td>
<td>8 (57%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>2 (9%)</td>
<td>7 (50%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
in particular correlated with the measured clinical parameters of CHF status (Table 2).

**Predictive Value of Biomarkers**

Each patient was followed up for 18 months, during which there were 16 CHF hospital admissions and 9 deaths. These events were analyzed with regard to the biomarker levels collected at the first visit. In subjects subsequently hospitalized for CHF, both NT-proBNP and SP-B were elevated (Figure 2). Similarly, for death, both NT-proBNP and SP-B were elevated (12 001 [3344, 40 584] versus 1419 [831, 3282], p=0.009, respectively). Because of cross-correlations between measured parameters, subjects in whom events occurred were matched with subjects free of events for clinical parameters in the following hierarchical order, on the basis of the strength of their association with events: NYHA class, DS (within 1 point), 6-MWT distance (within 30 m), age (within 8 years), and LVFS (within 1.5 points). On conditional logistic regression analysis, plasma SP-B as a continuous variable was independently associated with CHF hospitalization (OR, 1.00154; 95% CI, 1.00047, 1.00262) (p=0.005), whereas NT-proBNP added no further information over that gained from the clinical data (p=0.24) (Figure 3). Consequently, for each 1000-ng/mL increase in SP-B (which represents a 25% increase in the median value), an excess risk of 4.7-fold was noted. Because of small numbers, mortality analysis was not performed.

![Figure 1](image1.png)

**Figure 1.** Circulating NT-proBNP and surfactant protein-B levels in patients with CHF and control subjects. Data are box-and-whisker plots of (A) NT-proBNP (logarithmic scale) and (B) SP-B (linear scale) in n=53 patients with CHF and n=19 control subjects. Patients with CHF are divided into NYHA class II (n=17), class III (n=22), and class IV (n=14) patients. A, NT-proBNP was elevated in CHF (p<0.001) and was elevated in the class II CHF patient subgroup over the control group (tP=0.02). NT-proBNP increased sequentially within the CHF group as NYHA classification worsened (p<0.001). B, SP-B was elevated in patients with CHF (p<0.001) and was elevated in the class II CHF patient subgroup over the control group (tP=0.014). SP-B increased sequentially within the CHF group as NYHA classification worsened.*

![Figure 2](image2.png)

**Figure 2.** Circulating NT-proBNP and SP-B levels in patients hospitalized for CHF. Data are box-and-whisker plots of (A) NT-proBNP (logarithmic scale) and (B) SP-B (linear scale) in patients not hospitalized for CHF (n=37) and patients hospitalized for CHF (n=16) in the 18-month follow-up period. A, NT-proBNP was elevated in the subgroup hospitalized for CHF (*p=0.02). B, SP-B was elevated in the subgroup hospitalized for CHF (†p=0.002).

![Figure 3](image3.png)

**Figure 3.** Plasma SP-B and freedom from CHF hospitalization. Data are n=53 patients with CHF divided into tertiles of plasma SP-B levels (3395, 4509) (33rd and 66th percentiles). Kaplan-Meier curves depict freedom from CHF hospitalization after 18-month follow-up. Higher plasma SP-B was associated with higher rates of CHF hospitalization (p<0.001).

**TABLE 2. Correlations Between Measured Parameters in Patients With CHF**

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>DS</th>
<th>LVFS</th>
<th>6-MWT</th>
<th>SP-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP</td>
<td>r_s</td>
<td>-0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>r_s</td>
<td>0.25</td>
<td>-0.46*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVFS</td>
<td>r_s</td>
<td>-0.57*</td>
<td>0.64*</td>
<td>-0.46*</td>
<td></td>
</tr>
<tr>
<td>6-MWT</td>
<td>r_s</td>
<td>0.15</td>
<td>-0.39†</td>
<td>0.50*</td>
<td>-0.50*</td>
</tr>
<tr>
<td>SP-B</td>
<td>r_s</td>
<td>0.33</td>
<td>-0.30</td>
<td>0.40‡</td>
<td>-0.41‡</td>
</tr>
</tbody>
</table>

*p<0.001, †p<0.01, ‡p<0.05.
Change in Measured Parameters at the Time of Increased Loop-Diuretic Dose

During the follow-up period, there were 210 heart failure clinic assessments in the 53 patients; the frequency of clinic visits was at the discretion of the treating physician.

On 21 occasions, the treating physician elected to increase the loop-diuretic dosage, a decision made before blinded biomarker batch analysis. Comparisons of measured parameters at this clinic visit with those at the previous visit (median of 31 days previously) were consistent with clinical deterioration (Table 3), and there was an increase in plasma NT-proBNP (Figure 4A) and SP-B (Figure 4C).

Change in Measured Parameters After Increased Loop-Diuretic Dose

On 32 occasions, a clinic visit followed an increase in loop-diuretic dosage visit (median of 16 days previously). Comparison of parameters between these two visits was consistent with clinical improvement (Table 3), and there was a reduction in plasma NT-proBNP (Figure 4B) and SP-B (Figure 4D).

Discussion

Circulating surfactant protein-B levels are increased in ambulatory patients with CHF. Furthermore, plasma SP-B levels are related to CHF clinical status, and, like NT-proBNP, levels change within patients through episodes of decompensated CHF. The novel and unique ability of plasma SP-B to reflect the effect of raised Pmv on the lung is supported by its independent association with CHF hospitalization and suggests that SP-B may be a useful morbidity biomarker in CHF.

Surfactant Protein-B as a Biomarker in CHF

SP-B is uniquely suited to act as a biomarker of lung health.\textsuperscript{5,6} It is synthesized exclusively by type II alveolar epithelial cells\textsuperscript{5,6} and compartmentalized in the alveoli by apical secretion,\textsuperscript{6} such that the healthy lung maintains an epithelial lining fluid: plasma gradient of >1500:1.\textsuperscript{6} However, when the alveolocapillary barrier is damaged, SP-B is no longer effectively partitioned, and increased amounts leak into the bloodstream.\textsuperscript{5,6} Critical to its use as a biomarker, alveolar levels remain stable in lung disease,\textsuperscript{5,6} including acute cardiogenic pulmonary edema.\textsuperscript{16} Furthermore, in a rat model of CHF, we have previously demonstrated that increased plasma SP-B cannot be explained on the basis of increased alveolar SP-B levels.\textsuperscript{13} The short systemic half-life of SP-B (3 to 12 minutes)\textsuperscript{6} and its arteriovenous gradient suggests that plasma levels acutely reflect changes in lung permeability.\textsuperscript{6} Indeed, plasma levels closely reflect markers of lung function rather than hepatic or renal clearance markers.\textsuperscript{6,7}

Having previously demonstrated that surfactant protein-A leaks into the blood stream in acute respiratory distress

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**TABLE 3. Change in Clinical Parameters With Decision to Increase Diuretic**

<table>
<thead>
<tr>
<th>Heart Failure Clinic Visit</th>
<th>Heart Failure Clinic Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously Stable</td>
<td>Diuretic Increased</td>
</tr>
<tr>
<td>NYHA</td>
<td>3 (3, 3)</td>
</tr>
<tr>
<td>Weight</td>
<td>82 (61, 84)</td>
</tr>
<tr>
<td>DS</td>
<td>5 (4, 7)</td>
</tr>
<tr>
<td>LVFS</td>
<td>0.5 (0, 1.3)</td>
</tr>
<tr>
<td>6-MWT</td>
<td>343 (235, 436)</td>
</tr>
</tbody>
</table>

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**Figure 4.** Change in NT-proBNP and SP-B with CHF decompensation and after treatment. Data are circulating NT-proBNP levels in (A) n=21 patients with CHF at the previous stable clinic visit and at the clinic visit at which loop-diuretic dosage was increased (decompensated CHF), and (B) n=32 patients with CHF at the clinic visit at which loop-diuretic dosage was increased and at the follow-up clinic visit after treatment (logarithmic scale). C and D are SP-B levels (linear scale) in the same patient groups as A and B, respectively. Diamonds represent median values. A, There was an increase in NT-proBNP levels at the time of decompensated CHF (*P*<0.001). B, There was a decrease in NT-proBNP levels after treatment of decompensated CHF.* C, There was an increase in SP-B levels at the time of decompensated CHF. * D, There was a decrease in SP-B levels after treatment of decompensated CHF.*
syndrome,\textsuperscript{17} we postulated that SP-B would be an even better biomarker, given its smaller size.\textsuperscript{5} Therefore, effort was made to develop a technique for SP-B measurement in the blood.\textsuperscript{7} We had noted increased circulating SP-A\textsuperscript{7,17} and SP-B\textsuperscript{7,12} in acute cardiogenic pulmonary edema and consequently postulated that SP-B might be a useful biomarker of CHF. Although precisely how SP-B breaches the alveolocapillary barrier is uncertain,\textsuperscript{5,6} possibilities in the setting of CHF include mechanical barrier alteration such as high P_{mv}–induced “pore stretching”\textsuperscript{11} or “stress failure” of pulmonary capillaries.\textsuperscript{10} Other potential mechanisms include parenchymal inflation\textsuperscript{13} or altered protein barrier function secondary to parenchymal architectural changes seen in CHF.\textsuperscript{18} The latter is believed to explain the increase in plasma SP-A and SP-D in primary lung diseases in which there is alveolocapillary barrier remodeling, such as idiopathic pulmonary fibrosis.\textsuperscript{19,20}

**Relation of Circulating SP-B Levels to Severity of CHF**

SP-B increased with worsening NYHA classification. We believe this relation reflects raised P_{mv} and an associated increase in alveolocapillary permeability. Indeed, the results of the present study are consistent with our animal work, in which increased alveolocapillary barrier protein permeability was demonstrated in a rat model of CHF.\textsuperscript{13} Whereas lung fluid and dyspnea increase in response to high P_{mv}, elevated P_{mv} may also alter the integrity of the alveolocapillary barrier,\textsuperscript{21} allowing surfactant protein leakage into the circulation. This notion is supported by changes in SP-B levels within patients over time, through episodes of decompensated CHF. Because CHF decompensation results from elevated LV filling pressure, an increase in NT-proBNP was expected. We have shown for the first time that both NT-proBNP and SP-B levels change within individual patients over time, through episodes of CHF decompensation and successful outpatient treatment.

Whether circulating SP-B levels provide additive information to NT-proBNP levels in longitudinal CHF monitoring remains to be determined. However, physiologically, their modes of entry into the circulation are different, despite their positive association. NT-proBNP reflects LV wall stress and therefore the cause of elevated P_{mv}, whereas SP-B reflects the effects of elevated P_{mv} on the lung. This subtle distinction may be significant, given that the degree of pulmonary edema does not necessarily directly reflect central hemodynamics and therefore the absolute P_{mv} level. Indeed, lungs chronically exposed to high P_{mv} (CHF or mitral stenosis) undergo remodeling at the alveolocapillary barrier,\textsuperscript{18} protecting them from hydrostatic pulmonary edema at P_{mv} levels that would produce florid edema in lungs naive to raised P_{mv}.\textsuperscript{22} Our finding of an incremental association of SP-B with CHF hospitalizations highlights the differing release mechanism of these two biomarkers and the potential utility of a specific “morbidity” biomarker of lung health in CHF. Although SP-B is not specific for cardiac-induced alveolocapillary barrier damage and will be elevated in primary lung diseases such as pulmonary fibrosis and adult respiratory distress syndrome, it may provide complementary and independently useful information on clinical status in patients with CHF.

**Limitations of the Study**

Although the lack of hemodynamic data on the CHF cohort precludes confirmation that increased plasma SP-B in CHF reflects P_{mv}, the results are entirely consistent with our animal data in which plasma SP-B increased incrementally with LV end-diastolic pressure after myocardial infarction.\textsuperscript{13} Furthermore, because CHF is a chronic disease predominantly in outpatients, invasive hemodynamic measurement does not form part of modern standard care of these patients, making these measurements difficult to ethically justify in this study.

Because the subject numbers are small, further studies are needed to confirm our findings in CHF and document the day-to-day variation of plasma SP-B in these patients. To follow on from our findings of plasma SP-B fluctuations with clear clinical deterioration, a future prospective study of plasma SP-B in CHF is required to determine if this biomarker can predict the development of clinical CHF decompensation before its detection with clinical skills.

**Conclusions**

We have demonstrated for the first time increased circulating levels of SP-B in CHF and shown that levels are related to clinical status. We propose that plasma levels reflect altered alveolocapillary barrier integrity to protein secondary to raised P_{mv}. Indeed, the independent association of plasma SP-B levels with future CHF hospitalization and the documentation of changes in plasma SP-B levels through episodes of decompensated CHF support this position. SP-B warrants further investigation as a potentially clinically useful biomarker of CHF status.

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**References**


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