C-proSP-B: A Possible Biomarker for Pulmonary Diseases?

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Keywords

C-proSP-B · Biomarker · Pulmonary diseases · Interstitial lung diseases

Abstract

Background: Detection of surfactant proteins A and D (SP-A and SP-D) in the serum of patients with pulmonary diseases is thought to reflect an injury of the alveolar epithelial barrier and as such serve as a biomarker for these diseases. However, the data for SP-B are limited. **Objectives:** The aim of this feasibility study was to assess whether immature SP-B pre-proteins might have value as a possible biomarker for pulmonary diseases. **Methods:** In serum samples from patients with different chronic lung diseases (interstitial lung diseases [ILDs], chronic obstructive pulmonary disease, asthma, lung cancer, pulmonary hypertension, inflammation, patients on ventilator support; total $n = 283$), C-proSP-B was measured using an electrochemiluminescence immunoassay based on mouse monoclonal anti-C-proSP-B antibodies. Levels were correlated to lung functional and clinical param-

eters. **Results:** The highest C-proSP-B levels were detected in the serum of idiopathic pulmonary fibrosis (IPF) patients. In a multivariate analysis, C-proSP-B levels were able to discriminate IPF patients from patients with all other pulmonary diseases ($p < 0.0001$). No significant correlations were found between C-proSP-B levels and lung function, smoking history, or disease extent. **Conclusions:** SP-B pre-proteins might serve as a biomarker in pulmonary diseases with alveolar or interstitial damage such as ILDs, especially in IPF. Their role in the long-term monitoring of such diseases has to be clarified further.

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Introduction

Pulmonary diseases belong to the most common diseases worldwide. Some of them emerge acutely, others are chronic, and often they turn out to be progressive and lethal. Some pulmonary diseases present high heterogeneity in clinical behavior. Therefore, diagnosing and

managing the disease remains a challenge in daily practice and often requires a multidisciplinary approach between clinicians, radiologists, and pathologists. Furthermore, in many of them, early diagnosis may be disease modifying.

Biomarkers would be highly desired in many pulmonary diseases, but the search for potential candidates often lacks results applicable in daily clinical practice. At least in theory, molecular biomarkers have the potential to be used in several ways: as predisposition biomarkers, as diagnostic or screening tools, as a prognostic instrument, or as a therapeutic, predictive marker [1]. The ideal properties of such a biomarker would be high sensitivity and specificity for its intended purpose, easy and reliable collection of the sample from critically ill patients, easy measurement of the biomarker with reproducible results, and a defined role in pathogenesis [2]. So far, the continued interest in identifying and studying biomarkers has provided important information about the mechanisms involved in lung injury and repair and has also indirectly led to the generation of new ideas regarding potential novel therapeutic targets [2]. However, many of them lack sensitivity and specificity and therefore cannot be used as a clinical decision tool in the routine workup process.

In diseases in which hopes due to new therapeutic options arise [3, 4], predictive factors for the course of the disease and the response to therapy would be desirable. Therefore, the identification of reliable serum biomarkers would be helpful for both clinicians and patients [1].

Recent data have raised hope for the identification of plasma biomarkers for some pulmonary diseases. Examples for promising candidates are matrix metalloproteinases 1 and 7, collagens I and III, osteopontin, advanced glycosylation end product-specific receptor or serum chemokine (C-C motif) ligand 18, and KL-6/MUC1 [5–11]. However, while some value has been shown for most of these potential biomarkers, none has yet been proven to be of either diagnostic or prognostic significance.

In the ongoing engagement to discover biomarkers, alveolar epithelial cells are turning into the focus of research [12]. Maintenance of pulmonary function depends to a substantial extent on the function of type II alveolar epithelial cells that synthesize and secrete pulmonary surfactant lipids and proteins, thereby reducing the collapsing forces created at the air-liquid interface in the alveoli [13]. Lung epithelium-specific surfactant proteins are also able to leak across the alveolocapillary barrier into the circulation [14]. Mature surfactant protein B (SP-B) is usually secreted only through the apical surface of type

II alveolar epithelial cells into the alveoli, but can also reach the plasma by moving down its large concentration gradient from the alveoli into the blood when the alveolocapillary barrier is damaged [14, 15]. Plasma usually contains very little detectable SP-B. Since mature SP-B is normally intimately associated with alveolar surfactant phospholipids, it is possible that complexes so formed are too large to breach the alveolocapillary barrier. Alternatively, mature SP-B may breach the lung barrier, but is then rapidly degraded from the circulation or incorporated into cellular membranes [15]. SP-B precursor proteins are present in the endoplasmic reticulum, Golgi complex, and lamellar bodies, whereas mature SP-B is found only in lamellar bodies [16]. Proteolytic processing of the SP-B precursor into mature protein takes place intra- and extracellularly [16], resulting in the occurrence of the mature protein as well as the SP-B precursor and the processing intermediates in the alveolar epithelial lining fluid [17]. Because of differences in hydrophobicity, mature SP-B is intimately associated with the phospholipid-rich surfactant [18], whereas the less hydrophobic SP-B precursor and its processing intermediates (C-proSP-B and proSP-B) are less tightly bound to phospholipids and can therefore predominantly be recovered in the hydrophilic parts of, e.g., bronchoalveolar lavage fluid supernatant [17]. In addition, Agostoni et al. [19] stated that the immature form of SP-B seems to remain high in the blood for a longer time in patients with acute lung injury compared with mature SP-B. Therefore, one could expect that SP-B precursor proteins might have a role as biomarkers in diseases damaging the alveolocapillary barrier.

In the present prospective study, we aimed to investigate whether proSP-B and C-proSP-B have a potential role as a biomarker in pulmonary diseases, especially in those where the alveolocapillary barrier is substantially damaged [20–22]. Hence, we collected blood samples from a variety of patients with different pulmonary disease entities. The aim of this approach was to observe whether the different pulmonary diseases and their impact on the alveolocapillary barrier influence the blood level of the SP-B precursor proteins.

Materials and Methods

Study Design

A total of 283 patients with different pulmonary diseases were included in our study and serum samples were prospectively collected. In a pre-specified first study phase, which intended to detect short-term differences of the serum biomarker, blood samples were

Table 1. All patients included in this study categorized by disease, disease stage, demographic data, and lung function

Disease	Disease subgroup	Patients, <i>n</i>	Sex (M/F)	Age, years	VC _{max} , L	VC _{max} , % predicted	FEV ₁ , L	FEV ₁ , % predicted	FEV ₁ /VC _{max} , predicted
ILD (21)			12/9	63	2.6	72	2.1	74	85
	IPF	9							
	NSIP	4							
	sarcoidosis	6							
	collagenosis	1							
	UILD	1							
COPD (26)			14/12	66	2.5	71	0.8	33	38
	GOLD II	2							
	GOLD III	7							
	GOLD IV	17							
Asthma (19)			7/12	52	3.2	91	2.1	70	66
	intrinsic	9							
	extrinsic	4							
	undetermined	6							
Lung cancer (17)			10/7	64	2.6	78	1.9	69	72
	NSCLC stage I	2							
	NSCLC stage II	2							
	NSCLC stage III	6							
	NSCLC stage IV	7							
PAH (50)			21/29	62	2.9	88	2.2	82	78
	Dana Point I	32							
	Dana Point II	6							
	Dana Point III	2							
	Dana Point IV	10							
Inflammation (50)			23/27	58	2.4	71	1.3	58	65
	pneumonia	24							
	tuberculosis	10							
	non-CF bronchiectasis	16							
Ventilation (100)			58/42	64	2.5	78	1.7	68	67
	invasive	51							
	noninvasive	49							

Age and all lung function data are shown as medians. Figures in parentheses indicate number of patients. CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSCLC, non-small-cell lung cancer; NSIP, nonspecific interstitial pneumonia; PAH, pulmonary arterial hypertension; UILD, unclassifiable interstitial lung disease; VC, vital capacity.

collected from 123 patients at three different time points (days 1, 7, and 21). In a pre-specified second study phase, blood samples were taken at one time point only ($n = 160$) to enrich the cohorts of the first study phase. All samples were collected prospectively from in- and outpatients with different pulmonary diseases and distinctive disease stages (interstitial lung diseases [ILDs], chronic obstructive pulmonary disease [COPD], asthma, lung cancer, pulmonary inflammation, pulmonary hypertension, and patients under invasive and noninvasive ventilation) (Table 1). We selected patients with different stages of the respective disease to create a valuable cohort reflecting the wide variety of potential differential diagnoses and pulmonary comorbidities by purpose.

After assessment of total proSP-B/C-proSP-B values in both study phases, the highest values became apparent in patients with ILDs. We decided to group the patients in order to specifically

analyze the potential of proSP-B/C-proSP-B as a biomarker for ILDs and idiopathic pulmonary fibrosis (IPF) in particular as follows: Group I: all patients with pulmonary diseases except for ILD patients ($n = 262$). Group II: all ILD patients ($n = 21$). Group III: all ILD patients except for IPF patients ($n = 12$). Group IV: all IPF patients ($n = 9$).

Blood Samples

Serum for clinical testing was collected from patients using 9-mL Sarstedt Serum Z Monovette plastic blood collection tubes. After clot formation at room temperature, tubes were centrifuged at 1,300 g for approximately 20 min at room temperature. Aliquot vials with serum samples were stored at -70°C . Samples were shipped to a central testing site on dry ice and kept at -70°C until testing. All samples were tested in parallel.

Table 2. Lung function data of all patients with ILD

ILD group	VC _{max} , L	VC _{max} , % predicted	FEV ₁ , L	FEV ₁ , % predicted	VC _{max} /FEV ₁ , % predicted	DL _{CO} , % predicted
IPF	2.58	61.5	2.23	72.6	86.6	45
IPF	1.5	76	1.3	80	87	44
IPF	3.36	89.3	3.13	112.3	93.37	39
IPF	3.27	73.3	2.8	85	85.59	66
IPF	3.31	102.3	2.55	100.1	77.06	88
IPF	3.57	86.9	3.03	99.5	84.99	49
IPF	2.74	74.6	2.18	81.3	79.56	34
IPF	1.63	44.6	1.4	52.5	86.18	20
IPF	2.14	71.9	1.82	74.5	85.03	33
NSIP	0.79	31.9	0.73	36.7	92.16	n.a.
NSIP	1.28	34.3	1.14	41	89.51	18
NSIP	1.91	67.9	1.53	67.1	80.41	64
NSIP	1.97	48.7	1.59	52.7	80.51	34
Sarcoidosis	2.44	49.5	2.12	54.2	86.58	75
Sarcoidosis	3	105	2.6	106	85	89
Sarcoidosis	2.67	101.6	2.3	105.5	86.1	n.a.
Sarcoidosis	4.4	94	2.8	74	63	36
Sarcoidosis	2.67	54.4	1.15	29.7	42.86	49
Sarcoidosis	4	67	3.3	71	82	66
CTD-ILD	1.88	72.9	1.6	77.5	85.36	46
UILD	2.6	56	2	57	77	63

CTD-ILD, connective tissue disease-associated interstitial lung disease; DL_{CO}, diffusing capacity for carbon monoxide; FEV₁, forced expiratory volume in 1 s; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; UILD, unclassifiable interstitial lung disease; VC, vital capacity.

ProSP-B Assay Procedure

Measurement was based on an electrochemiluminescence immunoassay using a Tris(bipyridyl)ruthenium(II) complex as label. Mouse monoclonal anti-proSP-B antibodies (N- or C-terminus for proSP-B or C-proSP-B, respectively) were used as capture reagent, whereas mouse monoclonal anti-proSP-B (C-terminus) antibodies were used as detection reagent. In brief, the biotinylated capture antibody, the ruthenium-labeled detection antibody, and sample or standard material (10 µL) were incubated in homogeneous phase for 9 min at 37 °C. After the first 9 min streptavidin-coated beads were added, and binding of the formed immune complexes to the microparticles took place during a second 9-min incubation. After the second incubation, the reaction mixture was transferred into the measuring cell, where beads were captured to the electrode surface by a magnet. The measuring cell was washed to remove unbound label and filled with detection buffer containing Tris-propylamine. After applying voltage to the electrode, the emitted chemiluminescence light was detected by a photomultiplier. Results were determined via a two-point calibration curve. ProSP-B is given as ng/mL.

Statistical Methods

For inferential analyzes, *t* test and analysis of variance were used. For depiction of descriptive statistics, scatterplots, line plots, and boxplots were used. In boxplots, data outside the whiskers are

regarded as outliers. Within the box the median is depicted as a red line, the mean as a black line. The black lines with maximum extension connect the minimum and maximum data points (range). The study data were captured with MS Excel 2003 (clinical data). For data management and all analyses, the statistic software packages SAS 9.2 and JMP 8.0 (SAS Institute, Inc.) were used.

Results

Patients

A total of 283 patients (145 male, 138 female) with a mean age of 64 years were included. All demographic and clinical characteristics are detailed in Table 1. In summary, we collected specimens of a total of 26 patients with COPD (median forced expiratory volume in 1 s [FEV₁] 33%), 19 patients with asthma (median FEV₁ 70%), 17 patients with lung cancer, 50 patients with pulmonary hypertension, 50 patients with pulmonary inflammation (24 pneumonia, 10 tuberculosis, and 16 non-cystic fibrosis bronchiectasis with infection), as well as 100 patients of our interdis-

Table 3. All patients with ILD categorized by respective ILD with specific comorbidities, medications, and demographic data

ILD group	Comorbidities	Current ILD-specific therapy	Radiological pattern on HRCT	Smoking status	Age, years	Sex
IPF	CAD, arterial hypertension, obesity, dyslipoproteinemia, IDDM 2	none	UIP pattern	never	72	M
IPF	pneumonia	none	UIP pattern	never	79	F
IPF	DVT, pulmonary embolism	immunosuppression, NAC	UIP pattern	ex (10 PY)	70	M
IPF	DVT, PH, gout	none	UIP pattern	ex (40 PY)	69	M
IPF	hyperuricemia	none	UIP pattern	never	70	M
IPF	CAD, arterial hypertension, dyslipoproteinemia	none	UIP pattern	current (20 PY)	63	M
IPF	PH, CAD, PAOD, atrial fibrillation, arterial hypertension, dyslipoproteinemia	none	UIP pattern	ex (35 PY until 1974)	76	M
IPF	GERD, dysphagia, arterial hypertension	none	UIP pattern	never	75	M
IPF	osteoporosis, PH	none	UIP pattern	current	56	F
NSIP	PH, depression, hypertensive heart disease, arterial hypertension	none	NSIP pattern	n.a.	63	F
NSIP	IDDM 2	prednisolone/NAC	NSIP pattern	ex (25 PY)	67	M
NSIP	hypothyreosis	none	NSIP pattern	never	59	F
NSIP	none	prednisolone/NAC	NSIP pattern	never	58	M
Sarcoidosis	none	none	type IV	ex (15 PY)	39	M
Sarcoidosis	GERD	none	type III	ex (15 PY)	57	F
Sarcoidosis	none	none	type II	never	49	F
Sarcoidosis	none	prednisolone	type II	current (25 PY)	39	M
Sarcoidosis	none	prednisolone	type III	never	42	M
Sarcoidosis	GERD, obesity, bronchial hyperreagibility	none	type II	never	37	M
CTD-ILD	obesity hypoventilation syndrome, arterial hypertension, dyslipoproteinemia, gout	none	NSIP pattern	never	63	F
UIILD	none	none	subpleural, fibrotic	ex (10 PY)	58	M

CAD, coronary artery disease; CTD-ILD, connective tissue disease-associated interstitial lung disease; DVT, deep vein thrombosis; GERD, gastroesophageal reflux disease; HRCT, high-resolution computed tomography; IDDM, insulin-dependent diabetes mellitus; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NAC, N-acetylcysteine; NSIP, nonspecific interstitial pneumonia; PAOD, peripheral artery occlusive disease; PH, pulmonary hypertension; PY, pack-years; UIILD, unclassifiable interstitial lung disease; UIP, usual interstitial pneumonia.

plinary and pulmonary critical care units, of whom 49 were under noninvasive ventilation without sedatives and 51 under invasive, mechanical ventilation under general anesthesia. Patients with ILDs (total $n = 21$; subgroups: IPF $n = 9$; idiopathic nonspecific interstitial pneumonia [NSIP] $n = 4$; sarcoidosis $n = 6$; connective tissue disease-associated ILD $n = 1$; unclassifiable ILD $n = 1$) had a median age of 63 years, a median vital capacity of 2.6 L (72% predicted), and a median diffusing capacity for carbon monoxide of 49% predicted (all lung function parameters of ILD patients are depicted in Table 2). Only 1 patient with IPF was under immunosuppressive therapy with cyclophosphamide and prednisolone during the sampling. All other IPF patients were not under IPF-specific medication at the time of blood sampling. Two patients with NSIP were treated with prednisolone/N-acetylcysteine and 2 patients with sarcoidosis underwent treatment with oral prednisolone at the time of blood sampling. All other ILD patients in this study were naïve to specific ILD treatment before

recruitment ($n = 16$). Three patients were current smokers at the time point of inclusion, 7 were ex-smokers, and 9 were nonsmokers. The smoking status of 1 patient (NSIP) was not available. Four patients were not taking any drugs/medication on a regular basis. Relevant comorbidities and additional patient characteristics data are detailed in Table 3.

ProSP-B and C-proSP-B Longitudinal Observations

A correlation between treatment of the different diseases and proSP-B and C-proSP-B values could not be detected during the short-term, three-point monitoring (1, 7, and 21 days) in neither of the disease entities or during invasive or noninvasive respiratory support (see Fig. 1 for distinction of sex).

ProSP-B and C-proSP-B levels were also not significantly affected by sex (Fig. 1), age, or smoking status (data not shown). Furthermore, no significant differences between levels of proSP-B and C-proSP-B measurements

Fig. 1. Comparison of C-proSP-B in male and female interstitial lung disease patients over three time points. Within the box the median is depicted as a red line, the mean as a black line. The black lines with maximum extension connect the minimum and maximum data points (range). Data outside the whiskers are regarded as outliers.

could be determined at any point in time in any of the investigated disease entities. Therefore, further analyses focused only on C-proSP-B determinations.

C-proSP-B Levels

Since no significant longitudinal differences could be determined, data of day 1 in the first study phase were pooled with the values of the second study phase. The highest C-proSP-B levels were found in ILD (median 1,542 ng/mL, range 105–3,000 ng/mL, $n = 21$) compared to infectious diseases (median 635 ng/mL, range 101–5,291 ng/mL, $n = 50$), thoracic malignancies (median 447 ng/mL, range 42–1,807 ng/mL, $n = 22$), and pulmonary hypertension (median 451 ng/mL, range 26–3,000 ng/mL, $n = 50$). Low values were found in asthma (median 210 ng/mL, range 31–715 ng/mL, $n = 19$) and COPD (median 369 ng/mL, range 65–1,375 ng/mL, $n = 26$) (Fig. 2).

Differences between C-proSP-B levels in ILD patients (group II; mean log[C-proSP-B] = 6,895, $n = 21$) versus the pooled data of all other diseases excluding ILD (group

I; mean log[C-proSP-B] = 5,487, $n = 263$) were not statistically significant in a multivariate analysis ($p = 0.1148$).

C-proSP-B in ILD

In the group of ILD patients ($n = 21$), patients with IPF had a median C-proSP-B level of 2,222 ng/mL (range 1,530–3,000 ng/mL, $n = 9$). In 1 patient with connective tissue disease-associated ILD the level was 2,701 ng/mL, and in 1 patient with unclassifiable ILD it was 2,983 ng/mL. Patients with NSIP had a median C-proSP-B level of 1,757 ng/mL (range 1,213–2,774 ng/mL, $n = 4$), and patients with sarcoidosis had a median C-proSP-B value of 217 ng/mL (range 105–599 ng/mL, $n = 6$) (Table 4).

C-proSP-B Compared to Other Pulmonary Diseases

A multivariate analysis of C-proSP-B levels was able to discriminate IPF patients (group IV; mean log[C-proSP-B] = 7,682, $n = 9$) from patients with all other pulmonary diseases excluding ILD (group I; mean log[C-proSP-B] = 5,487, $n = 263$), i.e., asthma, COPD, lung cancer, pulmo-

Fig. 2. Comparative levels of C-proSP-B in a logarithmic scale comparing patients with idiopathic pulmonary fibrosis (IPF) versus patients with asthma, chronic obstructive pulmonary disease (COPD), lung cancer (LC), pulmonary hypertension (PH), and pneumonias (PNA) and those with invasive or noninvasive ventilation (IV-NIV). Data outside the whiskers are regarded as outliers.

Table 4. C-proSP-B levels in ng/mL in all ILD patients

ILD	Mean	Range
IPF (<i>n</i> = 9)	2,020	1,530–3,000
Sarcoidosis (<i>n</i> = 6)	217	105–599
NSIP (<i>n</i> = 4)	1,757	1,213–2,774
Unclassifiable (<i>n</i> = 1)	2,983	–
CTD-ILD (<i>n</i> = 1)	2,701	–

CTD-ILD, connective tissue disease-associated interstitial lung disease; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia.

nary hypertension, and pneumonia patients ($p < 0.0001$) as well as patients under invasive or noninvasive ventilation ($p < 0.0001$) (Fig. 2).

Discussion

Elevated serum levels, genetic variations, as well as insufficiencies of SP-B have been linked to a spectrum of both lung and cardiac diseases [19, 23–28]. It has been suggested that a chronic increase in the permeability of the lung parenchyma due to different pulmonary disease entities allows SP-B and its pre-proteins, which are normally almost not detectable in the serum of healthy subjects, to leak into the circulation [15]. Against this back-

ground, surfactant proteins may serve as ideal candidates as biomarkers in pulmonary diseases.

In this prospective feasibility study, we were able to demonstrate for the first time that C-proSP-B is significantly elevated in patients with IPF compared to patients with other chronic lung diseases. Whether this observation can be used to discriminate IPF from other forms of ILDs will have to be determined in a larger cohort of ILD patients.

The rationale for our study was that different lung diseases might cause differences in lung permeability for surfactant proteins in the alveolocapillary barrier. The primary aim of this feasibility study was to explore whether the serum levels of proSP-B and C-proSP-B were generally able to discriminate between different pulmonary diseases. For this, we prospectively collected specimens from a variety of patients with different lung diseases. Since a possible role of the mature SP-B is currently being discussed in chronic heart failure as well as in mechanical lung ventilation [22, 25, 27], we decided to include patients with pulmonary hypertension and patients who were under mechanical and noninvasive ventilation into our study to evaluate the possible influence of right heart failure as well as noninvasive and invasive ventilation on proSP-B/C-proSP-B levels.

According to expectations, C-proSP-B was readily detectable in the plasma of patients with different lung diseases. Interestingly, the levels of C-proSP-B were highest in patients with ILDs. In a multivariate analysis of

C-proSP-B levels, we were able to significantly discriminate IPF patients from all other patients with non-ILD pulmonary diseases. Although the route by which proteins leak through the alveolar capillary membrane is not yet fully understood, this could be associated with higher membrane permeability in ILDs due to regions of alveolar epithelial cell injury and hyperplasia, basal membrane disruption, as well as capillary leakage and subsequent aberrant wound healing known to be part of the disease mechanism in this disease entity. This is consistent with the already published data associating mature SP-B with acute lung injury [17].

Among the patients with ILD, patients suffering from pulmonary sarcoidosis were also included. In the ILD group the lowest values of C-proSP-B concentrations were measured in these patients, comparable to the results of asthma and COPD patients. Although the current literature suggests that there might be a correlation between elevated serum levels of mature SP-B and pulmonary sarcoidosis [29], our findings once more highlight the question whether the pathophysiological mechanisms underlying pulmonary sarcoidosis are comparable to those of other ILDs.

The high significance of elevated levels in IPF by no means precludes the significance of the findings in other lung disease entities analyzed. In these cases it would be very interesting to determine at which point in the evolution of the disease the C-proSP-B levels starts rising, and whether a potential reduction in levels could be utilized as a treatment monitoring biomarker. The 21 days for three determinations performed in part of the study population turned out to be too short a period in order to clarify this aspect.

A recently published study by Sin et al. [21] demonstrated that plasma proSP-B was significantly and independently associated with lung cancer and added to lung cancer prediction. We were not able to confirm these findings in our study, although it should be noted that all our patients were already diagnosed with lung cancer at the time of inclusion. A baseline comparison of (C-)proSP-B levels between the two studies is therefore not possible.

Another very recently published study by Leung et al. [20] analyzed the relationship between proSP-B levels and lung function decline in a large study population of smokers. They were able to demonstrate that circulating levels of proSP-B were associated with increased severity of airflow limitation and with rapid progression of COPD over 2 years in a large population of current and ex-smokers, reflecting the potential role of proSP-B as a prospec-

tive biomarker. Again, in our study model with only 21 days of follow-up a significant differentiation between C-proSP-B levels and lung function decline was not possible. Values for C-proSP-B in COPD and asthma patients were overall lowest in the pulmonary diseases tested here. This could be due to the pathophysiological differences between obstructive pulmonary diseases compared to the disease entities directly influencing the alveolocapillary barrier, such as ILDs or acute lung injury.

Magri et al. [27] discovered a relationship between mature SP-B circulating in plasma and heart failure. They were able to link severe alveolar gas diffusion damage to high serum levels of this protein. This led us to the hypothesis that the typical lung manifestations of an increased pulmonary pressure that ultimately results in an abnormal alveolar capillary gas diffusion and impaired lung mechanics (restriction) could have an influence on C-proSP-B levels. We collected blood specimens of 50 patients from different groups of pulmonary hypertension (WHO groups I–IV according to the Dana Point classification). The range of C-proSP-B levels varied significantly (26–3,000 ng/mL). Therefore, it was not possible to differentiate between the other investigated pulmonary diseases.

Di Marco et al. [22] lately reported that anesthesia, muscle paralysis, and a brief course of positive-pressure ventilation are associated with a significant increase in blood levels of the mature SP-B in a very short period of time (measurement 1 and 3 h after induction of anesthesia). In our study we did not observe elevated levels of C-proSP-B, neither in patients under invasive nor under noninvasive ventilation. This underscores the robustness of C-proSP-B as a prospective marker rather than a marker able to monitor short-term changes in potential membrane dysfunction of the alveoli.

In search of a biomarker for pulmonary diseases that would be specific, sensitive, and diagnostic, but also able to be noninvasively sampled, we investigated whether C-proSP-B could be a diagnostic aid for the definition and monitoring of different lung diseases. We designed this feasibility study to investigate whether there are significant differences in C-proSP-B values between different pulmonary diseases. The strength of our study lies in its prospective nature; however, there are several limitations that have to be addressed.

One limitation of our study is the lack of baseline values of C-proSP-B in a lung-healthy population. Furthermore, the observation period of 21 days is by far too short to proclaim C-proSP-B as a longitudinal marker. It will be necessary to conduct a study with both a larger sample size and a longer observation period to further investigate

our results. Finally, due to the small sample size of the ILD group, we were not able to discriminate statistically between IPF and other forms of ILDs.

Our findings with C-proSP-B underscore the conclusions of previous and recent literature that the precursor proteins of SP-B are particularly robust and adequate as potential prospective biomarkers for pulmonary diseases damaging the alveolocapillary barrier [17, 20, 21, 23, 28].

Conclusions

We were able to prove that particularly in patients with IPF, the high levels of C-proSP-B might be helpful in discriminating these patients from those with other pulmonary disease entities. Therefore, our data suggest a diagnostic potential of C-proSP-B as a biomarker in patients with IPF. Although the low number of IPF patients in this study limits the interpretation of our results, the role of C-proSP-B as a possible biomarker in IPF should be investigated further.

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Statement of Ethics

The institutional review board of Heidelberg University Hospital approved the data collection and analysis, and written informed consent was obtained from all patients before the procedure.

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