

Pulmonary Surfactant Protein B in the Peripheral Circulation and Functional Impairment in Patients With Chronic Heart Failure

Domingo A. Pascual-Figal,^a Jesús Sánchez-Más,^a Gonzalo de la Morena,^a Teresa Casas,^b Iris P. Garrido,^a Juan A. Ruipérez,^a and Mariano Valdés^a

^aUnidad de Insuficiencia Cardíaca, Servicio de Cardiología, Hospital Universitario Virgen de la Arrixaca, Departamento de Medicina Interna, Universidad de Murcia, Murcia, Spain

^bServicio de Bioquímica y Análisis Clínicos, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain.

Introduction and objectives. Surfactant protein B (SP-B) is a marker of damage to the alveolar-capillary barrier that could be useful for monitoring functional impairment in patients with chronic heart failure (HF).

Methods. Dyspnea-limited cardiopulmonary exercise testing was carried out in 43 outpatients with chronic HF (age 51[10] years, 77% male, left ventricular ejection fraction [LVEF] 33% [11%]). Peripheral blood serum samples were obtained at rest and during the first minute of peak exercise. The presence and concentration of SP-B in the serum samples were determined by Western blot analysis.

Results. At rest, SP-B was detected in 35 (82%) patients compared with only 6 (23%) healthy volunteers in a control group (n=26, age 51[10] years, 77% male). The median circulating SP-B level was higher in HF patients, at 174 [interquartile range, 70-283] vs 77 [41-152] ($P<.001$) in the control group. In HF patients, the presence of circulating SP-B was associated with a lower LVEF (31% [9.6%] vs. 41.8% [15%]; $P=.01$). Multivariate analysis showed that the resting SP-B level correlated with a greater VE/VCO₂ slope ($\beta=1.45$; $P=.02$). The peak-exercise SP-B level correlated almost perfectly with the resting level ($r=0.980$; $P<.001$), but there was no significant increase with exercise ($P=.164$). Nor was there a correlation with any other exercise parameter.

Conclusions. In patients with chronic HF, the level of pulmonary surfactant protein B in the peripheral circulation is increased and is correlated with ventilatory inefficiency during exercise, as indicated by the VE/VCO₂ slope.

Key words: Chronic heart failure. Exercise. Pulmonary function. Surfactant protein B.

Proteína surfactante pulmonar tipo B en circulación periférica y deterioro funcional en pacientes con insuficiencia cardíaca crónica

Introducción y objetivos. La proteína surfactante tipo B (PS-B) es un marcador de daño en la barrera alveolo-capilar y podría ser útil en la monitorización del deterioro pulmonar asociado a la insuficiencia cardíaca crónica (ICC).

Métodos. Se estudió a 43 pacientes ambulatorios con ICC (edad, 51 \pm 10 años; el 77% varones; fracción de eyección del ventrículo izquierdo [FEVI], 33% \pm 11%) a los que se realizó una prueba de esfuerzo cardiopulmonar limitada por disnea. Se obtuvieron muestras de sangre periférica en reposo y en el primer minuto tras el máximo esfuerzo. La presencia y la cantidad de PS-B en suero sanguíneo se analizaron mediante análisis *Western blot*.

Resultados. En reposo, se detectó PS-B circulante en 35 (82%) pacientes, frente a sólo 6 (23%) voluntarios sanos de una muestra control (n = 26; edad, 51 \pm 10 años; el 77% varones), con mayores concentraciones circulantes en pacientes con ICC (mediana [intervalo intercuartílico], 174 [70-283] frente al grupo control 77 [41-152]; $p < 0,001$). En pacientes con ICC, la presencia de PS-B circulante se asoció a una menor FEVI (31,4% \pm 9,6% frente a 41,8% \pm 15%; $p = 0,01$). Tras el ajuste multivariable, la cantidad de PS-B en reposo se correlacionó con una mayor pendiente VE/VCO₂ ($\beta = 1,45$; $p = 0,02$). Los valores de PS-B en el esfuerzo máximo se correlacionaron casi perfectamente con las cifras en reposo ($r = 0,980$; $p < 0,001$), pero no se incrementaron significativamente con el esfuerzo ($p = 0,164$) ni se correlacionaron con los parámetros de ejercicio.

Conclusiones. En pacientes con ICC, la proteína surfactante pulmonar tipo B está incrementada en la circulación periférica y se correlaciona con la ineficiencia ventilatoria en el ejercicio expresada como pendiente VE/VCO₂.

Palabras clave: Insuficiencia cardíaca crónica. Ejercicio. Función pulmonar. Proteína surfactante pulmonar tipo B.

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Correspondence: Dr. D.A. Pascual Figal.
Servicio de Cardiología. Hospital Universitario Virgen de la Arrixaca.
Ctra. Madrid-Cartagena, s/n. 30120 Murcia. España.
E-mail: dapascual@servicam.com

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ABBREVIATIONS

BNP: B-type natriuretic peptide
 HF: heart failure
 IQR: interquartile range
 SP-B: surfactant protein B

INTRODUCTION

The lack of clarity of the signs and symptoms in the diagnosis of heart failure (HF) has led to the search for new biomarkers. In recent years, B-type natriuretic peptide (BNP) has become accepted as a marker of cardiac function, and its diagnostic and prognostic value has been demonstrated in patients with chronic HF.¹⁻⁴ In these individuals, dyspnea produces a functional limitation and is usually the major symptom. The mechanisms of dyspnea on exertion in chronic HF are not fully understood, and they involve cardiac, pulmonary and peripheral vascular factors.⁵ The deterioration of pulmonary function is progressive throughout the course of the disease, and is associated with a poorer prognosis.⁶⁻⁹ However, to date, there is no known peripheral marker that, like BNP, aids us in monitoring lung function.

Surfactant protein B (SP-B) is found in the epithelium of the pulmonary alveolar-capillary membrane and is necessary for the maintenance of its function.¹⁰ The alveolar levels of SP-B remain constant, and it flows from the alveolus to the capillary blood as a consequence of a positive gradient, with rapid clearance from the capillary. If the alveolar-capillary barrier is damaged, SP-B leaks from the air space into the circulation as a result of the increased permeability.¹¹⁻¹³ This increased flow enables its detection in peripheral blood, and its measurement could be used as a sensitive, noninvasive method for monitoring pulmonary function in HF patients. In this respect, SP-B has been detected in the peripheral circulation of patients with acute pulmonary edema, as a consequence of the sudden increase in the pulmonary capillary pressure and the damage to the alveolar-capillary unit.¹⁴⁻¹⁶ In patients with chronic HF, the sustained increase in capillary pressure is associated with structural changes¹⁷⁻¹⁹ and, to date, only one study has suggested that the SP-B levels could correlate with the functional class and the prognosis in these patients.²⁰

The hypothesis of this study was that SP-B could be a marker of pulmonary function that would aid in the monitoring of functional deterioration in patients with chronic HF. The objectives of the present study are: *a*) to determine whether SP-B is detectable in patients with chronic HF, comparing

them to a control group of healthy volunteers; and *b*) to assess whether its presence correlates with functional parameters in a cardiopulmonary exercise test.

METHODS

Study Population

The study included 43 consecutive outpatients from a specialized HF unit, with an established diagnosis of chronic HF, who met the following selection criteria: *a*) exertional dyspnea as the major limiting symptom (New York Heart Association [NYHA] class II-III); *b*) an optimized medical treatment (including a beta-blocker and an angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist); *c*) a stable clinical condition, with no hospital admissions in the preceding 6 months; and *d*) not be diagnosed as having primary pulmonary disease and/or abnormal spirometry, defined as a forced expiratory volume in one second of less than 80% of the predicted value or a ratio of forced expiratory volume in one second to forced vital capacity of less than 70%. These criteria were established to achieve a homogeneous sample, without biases derived from conditions that could potentially affect the SP-B values.

All the procedures included in the study were carried out on the same morning: *a*) the patients had fasted since midnight the night before and, after a period of rest of 30 minutes, blood samples were collected from an antecubital vein; *b*) an echocardiographic study was performed (Sonos 5500, Philips, Andover, MA, USA); *c*) a symptom-limited cardiopulmonary exercise test was carried out; and *d*) within the first minute after the exercise test, a second blood sample was collected. We included an age and sex-matched control group (*n*=26) of asymptomatic healthy volunteers, who had no known history of disease or cardiovascular risk factors, and in whom the results of echocardiography and spirometry were normal. Under the same conditions with respect to rest and fasting as those of the group with chronic HF, a blood sample was obtained from each of the controls. This study was approved by the local ethics committee, and all the participants gave their written informed consent.

SP-B Determinations

The presence and amount of SP-B in serum was analyzed by means of Western blot. For each sample, 100 µg of total protein were incubated for 5 minutes at 95°C in Laemmli sample buffer and were separated

by means of electrophoresis under reductive conditions in 12% polyacrylamide gel. The gel was then transferred to a PVDF membrane (Immobilon-PSQ Membranes, Millipore, Bedford, MA, USA). A rabbit anti-PSB polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) diluted 1:750 and a horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Inc.) diluted 1:5000 were used as primary and secondary antibodies, respectively. For development, we employed the ECL Plus chemiluminescence kit (Amersham Pharmacia Biotech, Little Chalfont, UK) according to the manufacturer's instructions. Quantitative analysis was performed with the Gel-Pro Analyzer 3.1 software package (Sigma, St. Louis, MO, USA). The SP-B values are expressed as optical density units. A single specific band with an apparent molecular weight of 17 kDa was obtained, which corresponds to the active dimeric form of SP-B released from the bronchoalveolar barrier.²¹ The serum total protein concentration was determined by means of a bicinchoninic acid assay. The homogeneity of the total protein load in the different lanes of the gel was assessed by cutting the upper fourth of the membrane prior to blocking and staining it with 0.1% amido black dissolved in 45% methanol and 10% acetic acid. To verify the specificity of the immunoreactive bands, a pulmonary surfactant extract obtained from bronchoalveolar lavage performed in patients with primary lung tumors was utilized. The determinations were performed in triplicate in each sample. The plasma BNP concentration was measured in duplicate using a specific sandwich solid phase radioimmunoassay (ShionoRia BNP kit, CIS Bio International, Gif sur Yvette, France), as reported elsewhere.²²

Cardiopulmonary Exercise Test

All patients underwent a dyspnea-limited cardiopulmonary exercise test on a treadmill according to a modified Bruce protocol. Gas exchange and ventilation were assessed by means of a pneumotachograph equipped with a gas analyzer (CPX System, Medical Graphics Corp., St. Paul, MN, USA), which measured minute ventilation (VE), oxygen consumption (VO_2), and carbon dioxide consumption (VCO_2) every 10 seconds by means of a mass spectrometer (Amis 2000, Innovision, MedGraphics CardiO₂ System, Odense, Denmark). The patients were asked to exercise to exhaustion and all of them reached the anaerobic threshold and a respiratory quotient >1.05. Continuous monitoring with 12-lead electrocardiography was performed. Blood pressure was measured once every minute by means of a cuff sphygmomanometer. All the patients interrupted the test due to dyspnea; none of them

experienced chest pain or ischemic ST segment changes. The maximum VO_2 ($\text{VO}_{2\text{max}}$) was established as the value of greatest importance in the final phase of exercise, and was expressed in mL/kg/min and as a percentage (%) of the predicted normal level ($\%\text{VO}_{2\text{max}}$ or functional capacity). The predicted $\text{VO}_{2\text{max}}$ was calculated on the basis of sex, age, and weight.²³ The slope of the ratio of minute ventilation to carbon dioxide production (VE/VCO_2) was established as the coefficient of the ventilatory response to exercise or ventilatory inefficiency.²⁴

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution of quantitative variables. The SP-B and BNP values showed a deviation from normality and, thus, are expressed as medians (interquartile range [IQR]). The remaining quantitative variables were expressed as means (standard deviation [SD]) and the qualitative variables were expressed as number (%). For the comparison of the categorical variables, the χ^2 test and Fisher's exact test were used. The levels of SP-B in the group with chronic HF and in the control group were compared by means of the Mann-Whitney U test for independent samples. The SP-B levels at rest and during maximum effort were compared using the Wilcoxon test for paired samples. The association between SP-B levels and the diagnosis of chronic HF was studied by analyzing the receiver operating characteristic curve. For the study of the correlations, we used the logarithmic transformation of the levels of SP-B values (SP-B_{\log}) and BNP (BNP_{\log}) and simple linear regression analysis. The association between SP-B levels and the exercise parameters was evaluated by means of multiple linear regression analysis, adjusted for age, sex, left ventricular ejection fraction (LVEF), BNP and those variables with a $P < .1$ in the univariate analysis, and the β values and their 95% confidence intervals (95% CI) are provided. A P value less than .05 was considered significant. The statistical analysis was performed using a software package for social sciences (SPSS v. 15.0 for Windows, SPSS, Inc., Chicago, IL, USA).

RESULTS

Surfactant Protein B and Heart Failure

We studied a total of 43 patients with chronic HF whose clinical characteristics are shown in Table 1. The control group was made up of 26 healthy volunteers (age, 51.4 [9.6] years; 77% men). The presence of SP-B (Figure 1) was detected in the

TABLE 1. Clinical Characteristics

Age, mean (SD), y	51.2 (9.5)
Men, n (%)	33 (77)
Body mass index, mean (SD), kg/m ²	26.4 (4.0)
Ischemic etiology, n (%)	4 (9)
NYHA class, mean (SD)	2.4 (0.5)
Systolic arterial pressure, mean (SD), mm Hg	114 (20)
Heart rate, mean (SD), beats per minute	77 (13)
LVEF, mean (SD), %	33 (11)
End-diastolic volume, mean (SD), mL	194 (78)
E/A ratio, mean (SD)	1.10 (0.84)
Atrial fibrillation, n (%)	8 (19)
Complete left bundle branch block, n (%)	17 (40)
Furosemide, mean (SD), mg/d	48 (28)
Creatinine, mean (SD), mg/dL	1.34 (0.5)
Hemoglobin, mean (SD), g/L	13.3 (1.6)
BNP, mean [IQR], pg/mL	66.0 [25.7-185.2]
SP-B at rest, mean [IQR]	99.6 [19.7-241.0]
Cardiopulmonary exercise test	
VO ₂ max, mean (SD), mL/kg/min	17.7 (4.3)
Functional capacity, mean (SD), %VO ₂ max	56 (16)
VE/VCO ₂ slope, mean (SD)	35 (5)
Duration, mean (SD), seconds	318 (153)
Pressure 3 heart rate (max), mean (SD)	17320 (4308)
SP-B at peak exercise, mean [IQR]	77.4 [21.4-214.3]

BNP indicates B-type natriuretic peptide; IQR, interquartile range; LVEF, left ventricular ejection fraction; max, maximum; NYHA, New York Heart Association; SP-B, surfactant protein B; SD, standard deviation; VE, minute ventilation; VCO₂, carbon dioxide consumption; VO₂, oxygen consumption.

peripheral circulation of 35 HF patients (82%) versus 6 healthy volunteers (23%) ($P<.001$). In those with detectable SP-B, the concentration was higher in patients with HF: median [interquartile range, IQR] (interval), 174 [70-283] (13-1486) than in the healthy volunteers, 77 [41-152] (23-187) ($P<.001$) (Figure 2). The analysis of the receiver operating characteristic curve showed an area under the curve of 0.83 (95% CI, 0.72-0.91), and the detection of SP-B exhibited a sensitivity of 81%, a specificity of 77%, a positive predictive value of 86%, and a negative predictive value of 72% for the presence of HF. In the HF patients, the presence of circulating SP-B (vs its absence) was associated with a lower LVEF (31.4% [9.6%] vs 41.8% [15%]; $P=.010$). The SP-B levels did not correlate with any of the other clinical characteristics shown in Table 1 ($P>.05$ for all the analyses).

Surfactant Protein B and Functional Capacity

The SP-B_{log} value at the time of peak exercise exhibited a nearly perfect correlation with the resting SP-B_{log} value ($r=0.980$; $P<.001$), but there were no significant differences between the levels at these 2 times ($P=.164$). The exercise/resting SP-B ratio was 0.99 (0.23) [0.85-1.06] (0.44-1.65). In multiple

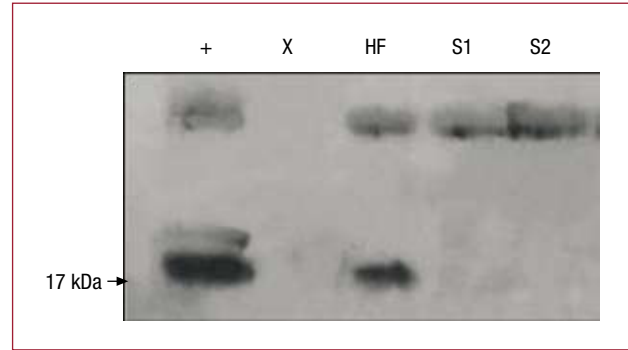


Figure 1. Detection of surfactant protein B (SP-B) using Western blot analysis: control of bronchoalveolar lavage of patients with primary lung tumors (+); patients with heart failure (HF); healthy volunteers (S1, S2); and blank lane (X).

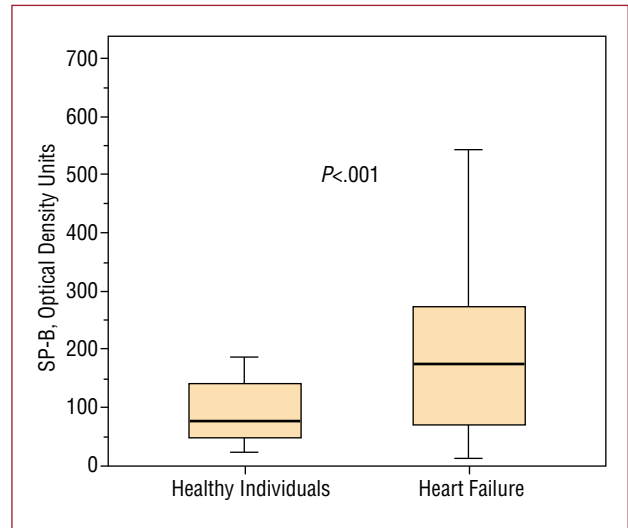


Figure 2 Quantification of circulating surfactant protein B (SP-B) levels in patients with heart failure and healthy controls.

linear regression analysis (Table 2), a higher SP-B_{log} level was the principal determinant of a lower functional capacity (lower %VO₂max), whereas age and SP-B_{log} at rest were positively associated with a greater ventilatory inefficiency (steeper VE/VCO₂ slope). There was no association between SP-B_{log} and the duration of exercise or the maximum arterial blood pressure × heart rate product, and the response of SP-B to exercise (exercise/rest ratio) exhibited no association with the VO₂max or the VE/VCO₂ slope ($P>.3$ for all the analyses).

DISCUSSION

The findings of this study have implications for the understanding of the pathophysiology of the

TABLE 2. Multiple Linear Regression Analysis

	Functional Capacity, %VO _{2max}		E/VCO ₂ Slope	
	β (95% CI)	P	β (95% CI)	P
SP-B _{log}	-1.7 (-7.1 to 3.6)	.25	1.45 (0.15 - 3.01)	.02
SP-B e/r	0.26 (-20.9 to 21.4)	.85	-2.11 (-8.5 to 4.3)	.55
BNP _{log}	-9.5 (-17.2 to 1.8)	.01	-1.35 (-1.17 to 3.87)	.23
Age	-0.36 (-0.88 to -0.55)	.30	-0.08 (-0.25 to 0.09)	.23
Sex	-7.2 (-18.8 to 4.4)	.12	-0.52 (-4.2 to 3.2)	.87

BNP indicates B-type natriuretic peptide; CI, confidence interval; e/r, exercise/rest ratio; LVEF, left ventricular ejection fraction; max, maximum; SP-B, surfactant protein B; VCO₂, carbon dioxide consumption; VO₂, oxygen consumption.

functional impairment in patients with chronic HF. Our work shows that SP-B is measurable and is present in a higher percentage and with higher levels in the peripheral circulation of HF patients than in that of a healthy population; likewise, the resting levels are correlated with ventilatory inefficiency during exercise (VE/VCO₂ slope). Moreover, the description for the first time of its measurement in human serum by Western blot increases the relevance of this study.

Previous reports have shown that SP-B is present in the peripheral circulation of healthy controls and, at a higher concentration, in different lung diseases, as an index of pulmonary status.^{10,25,26} In situations of acute lung damage, such as acute pulmonary edema or adult respiratory distress syndrome, SP-B has been found to be increased in peripheral blood as a consequence of the damage to the alveolar-capillary unit.^{14,27} Our study demonstrates that SP-B is present in the peripheral circulation of patients with stable chronic HF, at significantly higher levels than those detected in healthy individuals. Until now, only one study, by de Pasquale et al, has demonstrated the presence of SP-B in 53 HF patients at significantly higher levels than in 17 healthy controls.²⁰ The use in our study of Western blot for the detection of SP-B in human serum, rather than the enzyme-linked immunosorbent assay employed by de Pasquale et al, makes our work an important confirmatory study, as Western blotting is a more specific technique for the detection and comparison of protein levels. On the other hand, both studies show a wide range of levels, a circumstance that suggests a high interindividual variability, although the good correlation between the levels at rest and during exercise suggest a lower intraindividual variability.

In patients with HF, exercise is associated with an increase in pulmonary capillary pressures as well as pulmonary edema, which could play a role in an increase in the circulating SP-B.²⁸ However, in our

study, we did not observe a significant increase in SP-B during exercise. In 20 patients without HF who underwent a stress test for the diagnosis of heart disease, De Pasquale et al did not find an increase in SP-B in the overall study population, but did observe this effect in 10 patients in whom ischemia and changes in contractility were induced during exercise.²⁹ In our study, the detection of SP-B was associated with a lower resting LVEF. However, given the absence of echocardiographic or hemodynamic monitoring during exertion, we are unable to determine whether the induction of ventricular systolic dysfunction during exercise would help to identify a subgroup of patients with increased SP-B. On the other hand, the SP-B levels were measured immediately after exercise, and we have no data as to whether serial measurements would have produced significantly different results.

As a measure of the status of the alveolar-capillary unit, SP-B might explain the functional impairment due to dyspnea during exercise. In our study, SP-B was not predictive of the VO_{2max}, in contrast to BNP, which has been found to be a significant predictor of functional capacity.^{20,30} The VO_{2max} reflects the increase in cardiac output upon exercise, a fact that would explain its closer association with BNP, since the latter is a biomarker of cardiac function. Previously, in 53 patients with HF, De Pasquale et al found a correlation among SP-B values, NYHA functional class and the 6-minute walk test.²⁰ In contrast to that study, we found no correlation with more objective parameters such as the VO_{2max} and the %VO_{2max}. The inclusion in our study of stable patients with less functional impairment, as demonstrated by the low range of the baseline BNP and the relatively high VO_{2max} values, may have had an influence on the results obtained. However, we observed a modest, but significant, association with ventilatory inefficiency, reflected in a steeper VE/VCO₂ slope. In patients with HF, there is a progressive loss of capacity for the diffusion of gases

across the alveolar-capillary membrane, which, together with the increased congestion during exercise, implies a greater need for ventilation (VE) and a steeper VE/VCO₂ slope or ventilatory inefficiency.^{7,31} Guazzi et al³² demonstrated that the status of the alveolar-capillary membrane is the pulmonary parameter that correlates best with the VE/VCO₂ slope during exercise. This finding is in agreement with the results of our study, in which SP-B was associated with a steeper VE/VCO₂ slope.

Thus, our study suggests that SP-B, as a marker of alveolar-capillary damage, is measurable in the peripheral circulation of HF patients and is associated with the impairment of pulmonary function in terms of ventilatory inefficiency during exercise. Consequently, its measurement could be used as a pulmonary marker in HF and could be of prognostic value in these patients. Additional studies involving larger populations are necessary in order to better define its role in patients with HF.

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