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Opposite behavior of plasma levels surfactant protein type B and receptor for advanced glycation end products in pulmonary sarcoidosis

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Summary
Background: No biological marker is currently available for evaluating pulmonary involvement and/or for monitoring the clinical course of sarcoidosis. The present pilot study focused on possible relationships between circulating plasma levels of surfactant protein type B (SP-B) and plasma receptor for advanced glycation end products (RAGE) and lung function abnormalities in patients with pulmonary sarcoidosis, since both SP-B and RAGE have been previously suggested as lung injury markers. The plasmatic levels of these two proteins were also investigated with respect to functional capacity, as assessed by a cardiopulmonary exercise test (CPET).
Methods: Thirty pulmonary sarcoidosis outpatients and fifteen volunteers (Control Group) underwent lung function tests and CPET. Resting SP-B and RAGE plasma levels were also determined. Patients were then categorized according to the severity of their pulmonary involvement, as assessed in terms of lung diffusion for carbon monoxide (DL_{CO}) values.
Results: Group B showed SP-B levels higher and RAGE levels lower than Group A and Control Group ($p < 0.01$). Group A showed lower RAGE levels than Control Group ($p < 0.01$), whereas SP-B levels did not differ between these two groups. A significant univariate relationship was

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found between both SP-B and RAGE and several lung function data, particularly with DL_{CO} (SP-B Vs DL_{CO}: r: −0.437, $p = 0.016$; RAGE Vs DL_{CO}: r: −0.451, $p = 0.012$).
Conclusions: Circulating plasma levels of SP-B and RAGE showed an opposite behavior in patients with pulmonary sarcoidosis. SP-B values are directly related to alveolar unit damage, supporting a possible role of SP-B as a marker of disease severity in these patients. Differently, RAGE decreases in severe sarcoidosis, suggesting more complex underlying mechanisms.

Introduction

Total lung diffusion for carbon monoxide (DL_{CO}) is among the strongest functional parameters correlated with severity and prognosis in patients with pulmonary sarcoidosis [1,2]. Indeed, lung diffusion abnormality reflects the extent of the impairment of the entire gas exchange area, which can occur either in terms of capillary volume decrease or in terms of alveolar-capillary barrier thickness and/or ventilation-perfusion mismatch increase [2—5]. In pulmonary sarcoidosis, however, a broad spectrum of histopathologic abnormalities have been proposed to explain the typical DL_{CO} reduction, such as scattered granulomatous parenchymal involvement, hemosiderosis, pulmonary vein/artery granulomas, as far as pulmonary fibrosis [3,4,6,7].

Notwithstanding the above-mentioned structural lung damages, no plasmatic biomarker is currently reliable in evaluating pulmonary involvement and/or in monitoring the clinical course of pulmonary sarcoidosis [8]. In this context, both surfactant protein type B (SP-B) and plasma receptor for advanced glycation end products (RAGE) appear promising. Indeed, SP-B is one of the four surfactant proteins with a specific function in stabilizing the alveolar surfactant and, under physiological conditions, it is mainly confined into the alveolar space, being almost undetectable in bloodstream [9,10]. Conversely, in case of different pathological conditions characterized by an alveolar damage, its circulating plasma levels have been shown to increase [11—14]. On the other hand, RAGE belongs to the immunoglobulin superfamily, and its role in lung physiology still remains far from being elucidated. In fact, besides being recognized as a marker of acute lung injury [13—15], RAGE has also been suggested as a protective protein against the mechanisms underlying pulmonary fibrosis in different pulmonary diseases [16—18]. As a consequence, albeit with possibly different pathophysiological meanings, both circulating plasma levels of SP-B and RAGE might be useful in staging and managing patients with pulmonary sarcoidosis.

Therefore, the aim of this single-center pilot study was to investigate the relationships between circulating plasma levels of SP-B and RAGE and lung function abnormalities in patients with pulmonary sarcoidosis. Moreover, because resting lung function measurements might only partially reflect the real extent of lung impairment in pulmonary sarcoidosis [19,20], we also investigated the possible relationship between the plasmatic levels of these two proteins and exercise behavior, as assessed by a cardiopulmonary exercise test.

Methods

Study population

We evaluated 37 Caucasian outpatients with a biopsy specimen-proven sarcoidosis, who were regularly followed by a dedicated Respiratory Facility (pneumological ambulatory) of the Azienda Ospedaliera Sant'Andrea, "Sapienza" University of Rome. A cohort of eighteen apparently healthy volunteers, well-matched with the cases as for general characteristics, acted as controls.

All sarcoidosis patients had had chest radiographs and/or thoracic high-resolution computed tomography within a 6-month period, and they were accordingly classified [21]. Only patients with radiological evidence of pulmonary involvement, namely those with stage 2—4, were enrolled in the current study, whereas those with stage 0 and 1 were excluded. Other exclusion criteria were current smoke habit, moderate to severe renal failure, moderate to severe anemia, systemic or pulmonary arterial hypertension, diabetes, known coronary artery disease, echocardiographic evidence of left ventricular (LV) wall-motion abnormalities, depressed LV ejection fraction (LVEF $< 55\%$), valvular disease, or LV hypertrophy. Subjects who were receiving any cardiovascular therapy were also excluded.

All participants gave written informed consent to the procedures, and the study was approved by the internal review board of S. Andrea Hospital — "Sapienza" University of Rome (protocol number 0049-2010). Each subject who fulfilled the initial inclusion criteria underwent a full pneumological and cardiological clinical assessment in two sessions within the same day.

Specimen handling and assays

Immediately before starting clinical sessions, after lying down for 15 min, each participant underwent a venous blood sample drawing. Blood was collected in Vacutainer tubes containing citrate 0.129 mol/L, centrifuged at 3000 rpm at 4 °C, and plasma was stored at −80 °C for blind batch analysis.

The quantitative analysis of SP-B levels was performed by an enzyme-linked immunosorbent assay (Uscn Life Science Inc., Wuhan, China), as previously described [13,14]. The concentration of SP-B in the samples was then determined by comparing the absorbance of the samples to the standard curve and expressed as ng SP-B/mL. The limit of sensitivity was 1.95 ng/mL. Inter-assay coefficient of

variation was 11.6 ± 2.1%. Intra-assay coefficient of variation was 7.9 ± 1.5%.

Plasma levels of RAGE were determined using a commercially available enzyme-linked immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol [13,14]. Measurements were performed in duplicate and the results were averaged. The intra-assay and inter-assay coefficients of variation were <6 and <8%, respectively.

Resting pulmonary function evaluation

Participants were evaluated using standard resting pulmonary function tests (Quark PFT, Cosmed, Rome, Italy). Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and their ratios (FEV_1/FVC) were obtained according to the standard criteria [22]. Resting arterial blood gas levels, namely arterial O_2 (PaO_2) and carbon dioxide ($PaCO_2$) partial pressure, and arterial O_2 saturation (SaO_2) were measured. DL_{CO} was measured with the single-breath method, and its value was corrected for hemoglobin concentrations according to Cotes' formula [23].

Doppler echocardiography evaluation

All participants underwent a conventional M-mode and two-dimensional echocardiography Doppler evaluation (Acuson Sequoia® C512, Siemens Medical Solution, CA – USA). LV mass index (LVMI) was calculated with the standard formula: LVMI = LV mass divided by height to the power of 2.7. The LVEF was obtained using Simpson's biplane methods in two-dimensional echocardiography from the apical four-chamber view. The tricuspidal annular plane systolic excursion (TAPSE) was measured in the M-mode by placing the cursor through the lateral tricuspid annulus in the apical four-chamber view. Pulmonary systolic artery pressure (PASP) was indirectly obtained by adding the mean right atrial pressure to the peak systolic pressure gradient of tricuspid regurgitant flow [24]. Standard echocardiographic views were analyzed by a single expert physician blind to the results of subjects' clinical characteristics, and they were obtained according to the recommendations by the American Society of Echocardiography [25].

Cardiopulmonary exercise test (CPET)

A maximal symptom-limited CPET was performed on an electronically braked cycloergometer (Ergoline-800, Mortara, Bologna, Italy), the subject wearing a nose clip and breathing through a mass flow sensor (Quark PFT, Cosmed, Rome, Italy) connected to a saliva trap. A personalized ramp exercise protocol was performed, aiming at a test duration of 10 ± 2 min [26]. The exercise was preceded by few minutes of resting breath-by-breath gas exchange monitoring and by a 3-min unloaded warm-up. CPET was self-terminated by the subjects when they claimed that they had achieved maximal effort. However, we considered maximal effort as achieved if the respiratory exchange ratio (RER) was above 1.05.

Predicted values for pVO_2 were calculated according to the standard formula [27]. The anaerobic threshold was identified through a V-slope analysis of VO_2 and carbon dioxide production (VCO_2), and it was confirmed through specific behavior of O_2 (VE/VO_2) and CO_2 (VE/VCO_2) ventilatory equivalents and end-tidal pressure of O_2 and CO_2. The end of the isocapnic buffering period was identified when VE/VCO_2 increased and end-tidal pressure of CO_2 decreased. The relation between VE and VCO_2 (VE/VCO_2 slope) was calculated as the slope of the linear relationship between VE and VCO_2 from 1 min after the beginning of loaded exercise to the end of the isocapnic buffering period [28–30]. A 12-lead ECG, arterial systemic blood pressure, and arterial O_2 saturation (integrated pulse-oxymeter) were also recorded at baseline and during effort. All CPET were executed and analyzed by two physicians blinded to patients' clinical features.

Statistical analysis

An extension of the Shapiro–Wilk test of normality was preliminarily performed. Unless otherwise indicated, all data are expressed as mean ± SD. Statistical comparison was performed between controls and sarcoidosis patients, the latter being further subdivided into two subgroups according to the severity of their pulmonary involvement (Group A: DL_{CO} > 33rd percentile; Group B: $DL_{CO} \leq$ 33rd percentile). Categorical variables were compared with χ^2 test, while ANOVA followed by Bonferroni post-hoc analysis was used to compare the general characteristics and other continuous data between the three study groups. Univariate Pearson analysis was used to disclose, within the entire sarcoidosis group, possible relationships between pulmonary involvement severity, in terms of DL_{CO} values, and all the above-mentioned laboratory, pneumological, Doppler echocardiography and CPET data. A p value <0.05 was considered as statistically significant. All tests were two-sided. All data were evaluated with the database SPSS–PC + (SPSS–PC + Inc., Chicago, IL, USA).

Results

Out of the 57 subjects evaluated, only 30 sarcoidosis patients and 15 controls met the study inclusion/exclusion criteria and were actually considered in the present analysis. Two patients and two control subjects were excluded due to an exercise test judged as not maximal according to RER; one patient and one control subject were excluded due to poor compliance to DL_{CO} measurement techniques; finally, one patient was excluded due to echocardiographic evidence of left ventricular disease and another one for laboratory technical reasons. Diagnosis of sarcoidosis was proven by the presence of non-caseating, epithelioid-cell granulomas from mediastinal/peripheral lymph node biopsy in 16 patients, skin lesion biopsy in 4 patients and bronchial wall biopsy in the remaining patients.

Within the sarcoidosis group, 10 patients showed DL_{CO} values lower than 69% of the predicted value (lowest DL_{CO} tertile) and were included in Group B (stage 2: 1 patient; stage 3: 2 patients; stage 4: 7 patients); the remaining 20 patients, characterized by DL_{CO} values over the lowest tertile, constituted Group A (stage 2: 14 patients; stage 3: 6 patients; stage 4: none). At HRCT, available in all patients,

the occurrence of perilymphatic nodular thickening were found in almost all patients, areas of ground-glass attenuation in 11 patients (Group A: 3 patients, 15% Vs Group B: 8 patients, 80%) and intralobular interstitial thickening in 16 patients (Group A: 8 patients, 40% Vs Group B: 8 patients, 80%) whereas there were no signs of pulmonary arteries or venous compression in any case. Sarcoidosis involved at least one extrapulmonary organ in 11 of the 30 patients enrolled (skin: 4 patients; liver: 3 patients; joints: 3 patients; peripheral adenopathy: 4 patients; Group A: 6 patients, 30% Vs Group B 5 patients, 50%). As regards the possible previous or current specific treatment, 20 patients were receiving or had received prednisone (Group A: 13 patients, 65% Vs Group B: 7 patients, 70%), whereas 8 patients (Group A: 5 patients, 25% Vs Group B: 3 patients, 30%) had received methotrexate and/or cyclofosfamide treatment more than 6 months before enrolling in the study.

All three study groups were well-matched for age, gender, body surface area, arterial systemic blood pressure, and hemoglobin concentration, whereas the control group showed significantly lower values of resting heart rate than Group A and B. Doppler echocardiography showed a progressive increase in PASP values from control subjects to Group A and B. In Group B, TAPSE values were slightly reduced with respect to controls (Table 1).

As expected, Group B showed the worst pulmonary function in terms of FVC, FEV_1, and DL_{CO} values, while no difference was found for FEV_1/FVC and resting SaO_2. Both Groups A and B showed PaO_2 values lower than those of the control group. At CPET, lower pVO_2 values, higher VE/VCO_2 values and a more pronounced decrease in SaO_2 at peak exercise were present in Group B. Moreover, VE/VCO_2 values were also significantly higher in Group A than in healthy controls (Table 2).

Circulating plasma levels of SP-B were significantly higher in Group B than in healthy controls and Group A. Conversely, plasmatic RAGE concentrations were progressively lower from controls to Group A and Group B (Table 2, Fig. 1).

Univariate analysis disclosed a significant relationship of both SP-B and RAGE with several resting lung function and CPET data (Table 3). Circulating plasma levels of SP-B were related to DL_{CO} (Fig. 1), pVO_2, VE/VCO_2 slope, and ΔSaO_2. Circulating plasma levels of RAGE were related to DL_{CO} (Fig. 1), FVC, FEV_1, pVO_2, and ΔSaO_2.

Discussion

Our findings, albeit preliminary and obtained in a small cohort, showed for the first time a significant relationship between circulating plasma levels of SP-B and RAGE and lung function impairment in patients with pulmonary sarcoidosis. Interestingly, a relationship between DL_{CO} and plasma concentration of SP-B and RAGE was observed.

SP-B is one of the four surfactant proteins, and its specific function is to stabilize the alveolar surfactant. SP-B is exclusively synthesized in type II alveolar cells as an immature form that undergoes several proteolytic processes leading to an active form, namely the one evaluated in this study [9,10]. Under physiological conditions, this small peptide has a relevant gradient across the alveolar-capillary membrane, so that only a low SP-B concentration is found in the bloodstream, whereas, in case of damaged alveolar unit,

Table 1 Clinical characteristics in healthy controls and in the two subgroups of sarcoidosis patients according to the severity of pulmonary involvement (DLco values).

Variables	Healthy controls ($n = 15$)	Sarcoidosis patients		*P*-values ANOVA
		Group A ($n = 20$)	Group B ($n = 10$)	
Age, years	55 ± 8	54 ± 9	55 ± 13	Ns
Male, *n* (%)	6(40)	9(43)	4(40)	Ns
Body surface area, m^2	1.76 ± 0.14	1.88 ± 0.24	1.86 ± 0.19	Ns
NYHA class I/II/III, *n*	15/0/0[a,c]	17/2/1[c,d]	0/6/4	0.019
Haemoglobin, g/dL	14.0 ± 0.8	14.1 ± 0.9	13.8 ± 1.11	Ns
Non/ex/current smokers	14/1/0	18/2/0	9/1/0	Ns
Radiological stage, II/III/IV	—	14/6/0	1/2/7	—
Time from diagnosis, years	—	6.9 ± 5.3	5.9 ± 3.0	—
Systolic blood pressure, mmHg	121 ± 11	117 ± 9	114 ± 6	Ns
Diastolic blood pressure, mmHg	81 ± 7	75 ± 6	74 ± 8	Ns
HR, beats/min	78 ± 9[a,b]	84 ± 7	89 ± 8	0.023
LVEF, %	60 ± 4	61 ± 2	62 ± 5	Ns
LVMI, $g/m^{2.7}$	41 ± 16	43 ± 12	38 ± 8	Ns
TAPSE, mm	25 ± 3[c]	22 ± 3	19 ± 4	0.022
PASP, mmHg	22 ± 3[a,c]	29 ± 6[c,d]	32 ± 7	0.001

Data are expressed as mean ± SD or as absolute numbers (percentage). NYHA: New York Heart Association; HR: heart rate; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; TAPSE: tricuspid annular plane systolic excursion; PASP: echocardiographic pulmonary arterial systolic pressure.

[a] $p < 0.05$ Healthy controls Vs Group A.

[b] $p < 0.05$ Healthy controls Vs Group B.

[c] $p < 0.001$ Healthy controls and/or Group A Vs Group B.

[d] $p < 0.05$ Group A Vs Group B.

Table 2 Resting pulmonary function, CPET and laboratory data in healthy controls and in the two subgroups of sarcoidosis patients according to pulmonary involvement severity (DLco values).

Variables	Healthy controls ($n = 15$)	Sarcoidosis patients		*P*-values ANOVA
		Group A ($n = 20$)	Group B ($n = 10$)	
FVC, % predicted	107 ± 11[c]	103 ± 16[c]	69 ± 17	0.000
FEV_1, % predicted	103 ± 17[c]	103 ± 17[c]	67 ± 17	0.000
FEV_1/FVC, % predicted	101 ± 8	99 ± 7	95 ± 12	Ns
DL_{CO}, % predicted	93 ± 7[c]	91 ± 12[c]	50 ± 10	0.000
Resting PaO_2, mm Hg	90 ± 4[a,c]	83 ± 10	80 ± 9	0.024
Resting SaO_2, mm Hg	98 ± 1	96 ± 1	96 ± 1	Ns
pVO_2, ml/kg	25.1 ± 6.6[a,c]	20.8 ± 3.3[c]	14.3 ± 3.2	0.000
pVO_2, % predicted	98 ± 11[a,c]	86 ± 9[c]	58 ± 8	0.000
VE/VCO_2 slope	27.4 ± 3.0[a,c]	29.1 ± 3.7[c]	40.6 ± 5.5	0.000
R.E.R.	1.19 ± 0.06[a,c]	1.11 ± 0.05	1.13 ± 0.08	0.001
ΔSaO_2, %	0.6 ± 0.5[c]	1.1 ± 0.9[c]	6.1 ± 2.8	0.000
SP-B, ng/mL	3254 ± 1295[b]	3345 ± 1218[d]	5277 ± 2293	0.004
RAGE, pg/mL	1316 ± 274[a,c]	974 ± 341[d]	484 ± 165	0.000

Data are expressed as mean ± SD or as absolute numbers (percentage). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; DL_{CO}: total lung diffusion for carbon monoxide; PaO_2: arterial oxygen pressure; SaO_2: arterial oxygen saturation; pVO_2: peak oxygen uptake; VE/VCO_2 slope: relation between ventilation and carbon dioxide production; R.E.R.: respiratory exchange ratio; ΔSaO_2: (peak-resting SaO_2); SP-B: surfactant protein type B; RAGE: plasma receptor for advanced glycation end products.

[a] $p < 0.05$ Healthy controls Vs Group A.

[b] $p < 0.05$ Healthy controls Vs Group B.

[c] $p < 0.001$ Healthy controls and/or Group A Vs Group B.

[d] $p < 0.05$ Group A Vs Group B.

such as in case of acute pulmonary edema or lung injury, a high SP-B plasmatic concentration has been detected [11,31,32]. Accordingly, a possible role of SP-B as biomarker of alveolar-capillary barrier damage in patients undergoing transient mechanical ventilation [12,13] and in patients with stable chronic heart failure has also been proposed [14]. Similarly, in the present study, a higher plasma concentration of SP-B was detected in the sarcoidosis patients with the worst $DL_{CO,}$ suggesting the hypothesis that lung parenchymal and vascular destructive granulomatous lesions may result in an augmented SP-B leakage from the alveoli into the bloodstream. Indeed, DL_{CO} is strongly related to damages of alveolar-capillary unit, such as a decrease in capillary volume, or an increase in barrier thickness and/or in the ventilation-perfusion mismatch [2,4–7]. Accordingly, VE/VCO_2 slope and ΔSaO_2 values were significantly increased in the patients with the most impaired lung function, and they significantly correlated with circulating plasma levels of SP-B [14,29,33].

The observed behavior of RAGE underlines a different, much less clear pathophysiological meaning as compared to SP-B. RAGE belongs to the immunoglobulin superfamily and, whilst it is typically expressed at low levels under normal physiological conditions in most tissues, it exhibits a high basal level expression in lungs [16,17]. RAGE and SP-B have been previously suggested as possible biomarkers of lung injury [15,34]. Indeed, we showed an almost parallel increase in both the plasmatic levels of RAGE and SP-B in patients undergoing major surgery, and this finding was interpreted as a direct consequence of an alveolar damage due to the mechanical ventilation [13,14]. Conversely in a recent study, conducted on a cohort of healthy subjects with acute high altitude exposure, neither plasma SP-B nor RAGE levels increase have been found, this datum implying only an interstitial fluid overload rather than an acute damage in the alveolar cell barrier [35]. In the present study on sarcoidosis patients, the lowest RAGE values were found in those with the worst lung diffusion. As a matter of fact, a pivotal role of RAGE in protecting against the mechanisms underlying pulmonary fibrosis onset and progression has been suggested, given that it is involved in modulating the adhesion of alveolar epithelial cells to the basement membrane [16,17]. Indeed, fibrosis-like alterations have been found in the lungs of RAGE-deficient mice [18,36], and the presence of a significant inflammatory-induced RAGE down-regulation has been demonstrated in idiopathic pulmonary fibrosis [36,37]. Accordingly, given that the group with the lowest DLco values included a large percentage of patients with pulmonary fibrosis, it might be hypothesized that low RAGE values might be a cause, rather than a consequence, of the observed lung function impairment. However, evidence of a role of RAGE in promoting fibrosis has been supplied as well [38,39]. Therefore, it might be also speculated that our data simply reflect a loss of alveolar type I epithelial cells, primary expressers of RAGE. Other observations of the present study underlined a much less clear behavior of RAGE in patients with chronic lung damage such as those affected by sarcoidosis. Indeed, we reported a correlation of RAGE with DL_{CO} and with ΔSaO_2 during exercise but, unexpectedly and differently from SP-B, not with VE/VCO_2 slope, a marker of ventilation/perfusion mismatch during exercise. We presently do not have an explanation of the bulk of our findings, which, however, suggest that RAGE is not a direct marker of lung damage in sarcoidosis patients. Indeed, whilst RAGE mirrors alveolar unit damage in case of acute

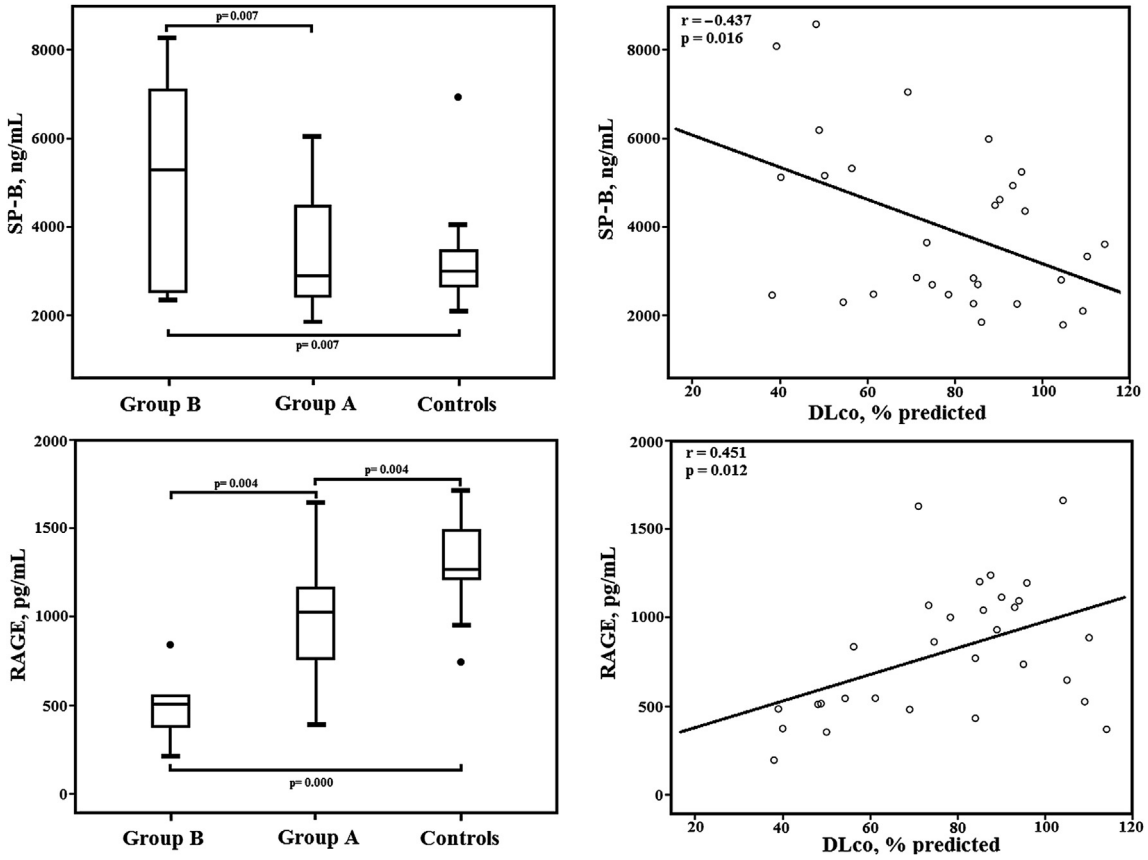

Figure 1 *Left panels*: differences in circulating Surfactant Protein type B (SP-B) (upper panel) and RAGE (lower panel) between healthy subjects (Controls) and patients with pulmonary sarcoidosis with lung diffusion for carbon monoxide (DL_{CO}) values higher (Group A) and equal/lower (Group B) than the lowest tertile. In the box plots, the central line represents the median distribution. Each box spans from 25th to 75th percentile points, and error bars extend from 10th to 90th percentile points. *Right panels*: Pearson univariate relationship within the entire sarcoidosis sample between SP-B (upper panel) and RAGE (lower panel) versus DL_{CO} (upper panel).

lung injury where the cell lysis leads to an increased leakage into the bloodstream, this seems not the case during chronic lung diseases, where its plasmatic concentration might be low due to a down-regulation and/or to an exhaustion of RAGE production. Furthermore, given that RAGE is known to be also secreted by monocyte/macrophage cells along the inflammatory cascade [40], it might be hypothesized that RAGE could increase in a manner proportional to number of active granulomas and, most likely, decrease when fibrosis replaces them. The actual study design clearly enables us only to speculate about mechanisms underlying our findings because of the small sample enrolled, the lack of patients without pulmonary involvement (stage 1) and, above all, because of we did not obtain seriate plasma RAGE dosages for each patient at different disease-steps.

Table 3 Relationship (univariate "*r*" values) between laboratory, main pneumological and cardiopulmonary exercise test data in the entire sarcoidosis study sample (*n*: 30).

Variables	DL_{CO}	FVC	FEV_1	pVO_2	VE/VCO_2 slope	ΔSaO_2	SP-B
FVC	0.696[b]	—	—	—	—	—	—
FEV_1	0.707[a]	0.905[a]	—	—	—	—	—
pVO_2	0.754[a]	0.747[a]	0.714[a]	—	—	—	—
VE/VCO_2 slope	−0.847[a]	−0.560[b]	−0.534	−0.688[a]	—	—	—
ΔSaO_2	−0.797[a]	−0.608[a]	−0.648[a]	−0.700[a]	0.682[a]	—	—
SP-B	−0.437[c]	Ns	Ns	−0.356[c]	0.450[c]	0.361[c]	—
RAGE	0.451[c]	0.502[b]	0.429[c]	0.563[b]	Ns	−0.531[b]	Ns

For abbreviations see Table 1.
[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

In conclusion, this is the first study where circulating plasma levels of SP-B and RAGE were measured in patients with pulmonary sarcoidosis. We showed a significant relationship of the plasmatic levels of these two proteins with several lung function measurements routinely adopted in the clinical management of this setting of patients. Longitudinal studies in larger study samples are needed to substantiate our brief report, mainly concerning a possible utility of plasma SP-B levels in the daily clinical management as well as its hypothetical prognostic role in patients with sarcoidosis. Differently, our data about RAGE behavior call for more dedicated experimental studies in order to elucidate its role in this setting of patient, mainly focusing on the relationship between plasma RAGE levels with the effective RAGE expression in the lung.

Conflict of interest

There are no conflicts of interest in connection with the submitted article.

References

[1] Iannuzzi RC, Rybicki Ba, Teirstein AS. Sarcoidosis. N Engl J Med 2007;357:2153—65.

[2] Sue DY, Oren A, Hansen JE, Wasserman K. Diffusing capacity for carbon monoxide as a predictor of gas exchange during exercise. N Engl J Med 1987;316:1301—6.

[3] Takemura T, Matsui Y, Saiki S, Mikami R. Pulmonary vascular involvement in sarcoidosis: a report of 40 autopsy cases. Hum Pathol 1992;23:1216—23.

[4] Lamberto C, Nunes H, Le Toumelin P, Duperron F, Valeyre D, Clerici C. Membrane and capillary blood components of diffusion capacity of the lung for carbon monoxide in pulmonary sarcoidosis. Relation to exercise gas exchange. Chest 2004;125:2061—8.

[5] Magrì D, Agostoni P, Ricotta A, Pisani L, Cauti FM, Onofri A, et al. NT-proAtrial Natriuretic Peptide as a possible biomarker of cardiopulmonary involvement in sarcoidosis. Eur J Intern Med 2013;24:278—84.

[6] Emirgil C, Sobol BJ, Herbert WH, Trout K. The lesser circulation in pulmonary fibrosis secondary to sarcoidosis and its relationship to respiratory function. Chest 1971;60:371—8.

[7] Nunes H, Humbert M, Capron F, Brauner M, Sitbon O, Battesti JP, et al. Pulmonary hypertension associated with sarcoidosis, haemodynamics and prognosis. Thorax 2006;61:68—74.

[8] Tzouvelekis A, Kouliatsis G, Anevlavis S, Bouros D. Serum biomarkers in interstitial lung diseases. Respir Res 2005; 21(6):78.

[9] Goerke J. Pulmonary surfactant: functions and molecular composition. Biochim Biophys Acta 1998;1408:79—89.

[10] Serrano AG, Perez-Gil J. Protein—lipid interaction and surface activity in the pulmonary surfactant system. Chem Phys Lipids 2006;141:105—18.

[11] De Pasquale CG, Arnolda LF, Doyle IR, Grant RL, Aylward PE, Bersten AD. Prolonged alveolocapillary barrier damage after acute cardiogenic pulmonary edema. Crit Care Med 2003;31: 1060—7.

[12] Magrì D, Brioschi M, Banfi C, Schmid JP, Palermo P, Contini M, et al. Circulating plasma surfactant protein type B as biological marker of alveolar-capillary barrier damage in chronic heart failure. Circ Heart Fail 2009;2:175—80.

[13] Agostoni P, Banfi C, Magrì D, Vignati C, Doria E, Salvioni E, et al. Kinetics of plasma SP-B and RAGE during mechanical ventilation in patients undergoing major vascular surgery. Respir Physiol Neurobiol 2011;178:256—60.

[14] Agostoni P, Banfi C, Brioschi M, Magrì D, Sciomer S, Berna G, et al. Surfactant protein B and RAGE increases in the plasma during cardiopulmonary bypass: a pilot study. Eur Respir J 2011;37:841—7.

[15] Uchida T, Shirasawa M, Ware LB, Kojima K, Hata Y, Makita K, et al. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. Am J Respir Crit Care Med 2006;173:1008—15.

[16] Mukherjee TK, Mukhopadhyay S, Hoidal JR. Implication of receptor for advanced glycation end product (RAGE) in pulmonary health and pathophysiology. Respir Physiol Neurobiol 2008;162:210—5.

[17] Buckley ST, Ehrhardt C. The receptor for advanced glycation end products (RAGE) and the lung. J Biomed Biotechnol 2010. 917108.

[18] Queisser MA, Kouri FM, Konigshoff M, Wygrecka M, Schubert U, Eickelberg O, et al. Loss of RAGE in pulmonary fibrosis: molecular relations to functional changes in pulmonary cell types. Am J Resp Cell Mol Biol 2008;39:337—45.

[19] Risk C, Epler GR, Gaensler EA. Exercise alveolar—arterial oxygen pressure difference in interstitial lung disease. Chest 1984;85:69—74.

[20] Hsia CC. Recruitment of lung diffusing capacity: update of concept and application. Chest 2002;122:1774—83.

[21] Statement of sarcoidosis: joint statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999;160:736—55.

[22] Official statement of the ATS: standardization of spirometry. Am J Respir Crit Care Med 1995;152:1107—36.

[23] Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity): report Working Party Standardization of Lung Function Tests, European Community for Coal and Steel; official position of the European Respiratory Society. Eur Respir J Suppl 1993;16:41—52.

[24] Sciomer S, Magrì D, Badagliacca R. Non-invasive assessment of pulmonary hypertension: Doppler-echocardiography. Pulm Pharmacol Ther 2007;20:135—40.

[25] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, , et alChamber Quantification Writing Group, American Society of Echocardiography's Guidelines and Standards Committee, European Association of Echocardiography. Recommendation for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standard Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Society of Echocardiograph, a brunch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440—63.

[26] Agostoni P, Bianchi M, Moraschi A, Palermo P, Cattadori G, La Gioia R, et al. Work-rate affects cardiopulmonary exercise test results in heart failure. Eur J Heart Fail 2005;7: 498—504.

[27] Wasserman K, Hansen JE, Sue DY, Casaburi R, Whipp BJ. Principles of exercise testing and interpretation. 4th ed. Baltimore: Lippincott Williams & Wilkins; 2005. p. 10—65.

[28] Piepoli MF, Corrà U, Agostoni PG, Belardinelli R, Cohen-Solal A, Hambrecht R, , et alTask Force of the Italian Working Group on Cardiac Rehabilitation Prevention, Working Group on Cardiac Rehabilitation and Exercise Physiology of the European Society of Cardiology. Statement on cardiopulmonary exercise testing in chronic heart failure due to left ventricular dysfunction: recommendation for performance and

interpretation. I. Definition of cardiopulmonary exercise testing parameters for appropriate use in chronic heart failure. Eur J Cardiovasc Prev Rehabil 2006;13:150—64.
[29] Bussotti M, Magrì D, Previtali E, Farina S, Torri A, Matturri M, et al. End-tidal pressure of CO_2 and exercise performance in healthy subjects. Eur J Appl Physiol 2008;103:727—32.
[30] Agostoni P, Valentini M, Magrí D, Revera M, Caldara G, Gregorini F, et al. Disappearance of isocapnic buffering period during increasing work rate exercise at high altitude. Eur J Cardiovasc Prev Rehabil 2008;15:354—8.
[31] Bersten AD, Hunt T, Nicholas TE, Doyle IR. Elevated plasma surfactant protein-B predicts development of acute respiratory distress syndrome in patients with acute respiratory failure. Am J Respir Crit Care Med 2001;164:648—52.
[32] De Pasquale CG, Arnolda LF, Doyle IR, Aylward PE, Russell AE, Bersten AD. Circulating surfactant protein-B levels increase acutely in response to exercise-induced left ventricular dysfunction. Clin Exp Pharmacol Physiol 2005;32:622—7.
[33] Kleber FX, Vietzke G, Wernecke KD, Bauer U, Opitz C, Wensel R, et al. Impairment of ventilator efficiency in heart failure: prognostic impact. Circulation 2000;101:2803—9.
[34] Calfee CS, Budev MM, Matthay MA, Church G, Brady S, Uchida T, et al. Plasma receptor for advanced glycation end-products predicts duration of ICU stay and mechanical ventilation in patients after lung transplantation. J Heart Lung Transplant 2007;26:675—80.
[35] Agostoni PG, Swenson ER, Fumagalli R, Salvioni E, Cattadori G, Farina S, et al. Acute high-altitude exposure reduces lung diffusion: data from the HIGHCARE Alps project. Respir Physiol Neurobiol. http://dx.doi.org/10.1016/j.resp.2013.04.005; 2013.
[36] Englert JM, Hanford LE, Kaminski N, Tobolewski JM, Tan RJ, Fattman CL, et al. A role for the receptor for advanced glycation end products in idiopathic pulmonary fibrosis. Am J Pathol 2008;172:583—91.
[37] Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One 2007;2:e482.
[38] Bargagli E, Penza F, Bianchi N, Olivieri C, Bennett D, Prasse A, et al. Controversial role of RAGE in the pathogenesis of idiopathic pulmonary fibrosis. Respir Physiol Neurobiol 2009;165:119—20.
[39] Chen L, Wang T, Wang X, Sun BB, Li JQ, Liu DS, et al. Blockade of advanced glycation end product formation attenuates bleomycin-induced pulmonary fibrosis in rats. Respir Res 2009;10:55.
[40] Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest 2001;108:949—55.