Bronchoalveolar lavage immunoglobulin A and G and antiproteases correlate with changes in diffusion indices during the natural course of pulmonary sarcoidosis

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ABSTRACT: We wanted to determine whether cell populations and soluble components in bronchoalveolar lavage (BAL) could be useful in predicting the outcome of lung function and chest radiography in patients with untreated pulmonary sarcoidosis.

Analysis of soluble proteins in BAL fluid, included the levels of immunoglobulins and the two major antiproteases, alpha₁-macroglobulin (α_2 -M) and alpha₁-protease inhibitor (α_1 -PI), expressed as a relative coefficient of excretion (RCE). Thirty one nonsmoking patients with biopsy proven sarcoidosis, who remained untreated, had reassessment of lung function tests after 6–54 months (median 21 months).

No correlation was observed between initial BAL data and changes in lung volumes and radiographic opacities. By contrast, the initial BAL immunoglobulin A and G (IgA and IgG) RCE correlated inversely with the change in transfer factor for carbon monoxide (TLCO) in the whole group and in patients with sarcoidosis of recent origin (estimated disease duration <6 months). In the whole group and in patients with longstanding disease (estimated disease duration >24 months, or radiographic Stage 4), the change in carbon monoxide transfer coefficient (Kco) correlated negatively with the initial α_1 -PI RCE and positively with the initial helper to suppressor T-cell (T4/T8) ratio. By contrast, no significant difference in BAL cellular and protein data was found between patients with recent and longstanding sarcoidosis.

We conclude that: 1) among the BAL cellular and protein parameters investigated, immunoglobulins, antiproteases and T4/T8 ratio are predictive markers for the changes of diffusion indices in patients with untreated sarcoidosis; and 2) the duration of disease at the time of the BAL procedure should be taken into account when assessing the predictive value of BAL parameters

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Pulmonary sarcoidosis is characterized by a mononuclear cell alveolitis and noncaseating granulomas associated with various degree of interstitial fibrosis. Current concepts on the pathogenesis of the disease suggest that a T-lymphocyte alveolitis, predominantly of the helper-induced phenotype, precedes and mediates the development of granulomas and fibrosis [1]. Only a minority of affected patients will develop progressive impairment of their pulmonary function leading to significant morbidity [2]. Therefore, the major problem in the management of pulmonary sarcoidosis is to identify those patients whose lung function is likely to deteriorate.

Since the cells recovered in bronchoalveolar lavage (BAL) fluid reflect the alveolitis [3], the analysis of BAL cell populations, especially lymphocyte count and T-cell

subsets, has largely been used to define prognostic markers [4–16]. Some investigators reported that a high BAL lymphocyte count was associated with a higher risk of pulmonary function deterioration [4, 9] and with a better response to steroid therapy [7], whilst others found that the helper to suppressor T-cell ratio (T4/T8) was more reliable [5, 8]. However, these promising data have been largely tempered by several other reports [6, 10–16]. The discrepancies between the results of these studies could be related to differences in patient selection with regard to race, smoking habits, previous treatment, and the duration of disease at the time of assessment [12, 14, 17, 18]. On the other hand, it is likely that the absolute number or percentage of BAL cell types or even the phenotypic subpopulations provide only rough information on their functional status, and hence on the

potential deleterious effect of their secretory products on the lung. Therefore, a number of these secretory products have been investigated and related to disease activity [1]. In this regard, we recently reported, in a cross-sectional study, that immunoglobulin A and G (IgA and IgG) levels in BAL fluid correlated with defects in pulmonary gas exchanges [19]. We also suggested that α_2 -macroglobulin (α_2 -M), a major antiprotease and a marker of inflammatory processes, could represent a sensitive index of the alveolitis [20, 21]. However, the usefulness of these soluble components of BAL fluid has not, so far, been evaluated in predicting the outcome of sarcoidosis.

The main purpose of this study was to assess the predictive value of BAL proteins (immunoglobulins and antiproteases) as compared to cell populations with respect to the changes in lung function during the natural course of pulmonary sarcoidosis. Moreover, we evaluated whether the predictive value of these BAL indices could be influenced by the duration of disease.

Materials and methods

Subjects

We included 31 consecutive patients with biopsy proven sarcoidosis, who fulfilled the following criteria: 1) nonsmoker; 2) no steroid treatment for the preceding 3 yrs; 3) available for a follow-up period of at least 6 months; and 4) no steroid treatment during the follow-up period. Among this group of 31 patients, one patient with uveitis was included since he refused steroid treatment, and nine subjects were referred to our institution with the diagnosis of pulmonary sarcoidosis previously established. However, the first assessment in our institution, including standard pulmonary function tests, chest roentgenograms and BAL, was considered as the "baseline" assessment. The outcome was assessed by the results of pulmonary function tests at the end of the follow-up period (median 21 months; range 6–54 months). During the follow-up, a complete clinical, radiological and functional recovery was observed in six patients (four patients with a disease duration <6 months, and two patients with a disease duration of 7–24 months; four with a radiographic Stage 1, and two with a Stage 2). During the study, 10 additional patients meeting the above criteria 1–3 were seen in our centre and treated with corticosteroids. Since no strict protocol of steroid therapy was applied, these patients were excluded from the present study. All investigations performed during the study, including BAL procedures, were approved by the Local Ethics Committee.

Radiology

Chest roentgenograms were classified according to Scadding [22] as: Stage 1 (hilar lymphadenopathy alone); Stage 2 (parenchymal infiltrates with lymphadenopathy); Stage 3 (parenchymal infiltrates without

lymphadenopathy); or Stage 4 (pulmonary "distortion" including hilar retraction, atelectasia, bullae and fibrosis). The severity (profusion) of parenchymal opacities was graded on a 10 point scale, modified from the ILO/UC classification according to McCloud *et al.* [23]. All chest X-ray films were read by two observers who were experienced in the use of the ILO/UC scoring system, and who were unaware of patients records.

Lung function

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were obtained by standard spirometry. Total lung capacity (TLC) was measured in a body plethysmograph (Jaeger). Transfer factor for carbon monoxide (TLCO) and correction for alveolar volume (the transfer coefficient, Kco) were determined using the Cotes single-breath method. Results were expressed as the percentage of predicted values taken from the European Community for Coal and Steel standard tables [24]. The evolution of pulmonary function tests at the end of the follow-up period was expressed as the percentage increase (positive values) or decrease (negative values) as compared to the corresponding initial values.

Bronchoalveolar lavage

BAL was performed according to a standardized protocol [19–21]. Briefly, after local anaesthesia of the upper respiratory tract, the fiberoptic bronchoscope was securely wedged in a subsegment, either of the right middle lobe or the lingula; 200 ml of sterile 0.9% saline solution were instilled in four aliquots and, thereafter, gently aspirated. The first aliquot (bronchial lavage) was discarded and the studies were performed on a pool of the following three aliquots. The recovered fluid was filtered through a single layer of gauze to remove gross mucus, and an aliquot was saved for total and differential cell count. The lavage was then centrifuged at $500\times g$ for 10 min and the cell pellet was used in lymphocyte subpopulation studies, whilst the supernatant was kept at $-40^\circ C$ for protein analysis.

Cellular studies

Cell differentials were performed on cytopsin preparations using the same centrifuge (Cytospin 1) at the same speed to minimize artificial variations between different samples. The lymphocyte subpopulations in BAL and blood (obtained on the day of the lavage) were determined with the fluorescent monoclonal antibodies (fluorescein isothiocyanate (FITC)) OKT4 (helper) and OKT8 (suppressor) according to REINHERZ *et al.* [25]. The cell-bound fluorescence in the lymphocyte population was measured using an Epics C Coulter flow cytometer. Data were expressed as percentage positive (OKT4, OKT8) cells in the total lymphocyte population.

Protein assays

Serum levels of albumin, IgG, IgA, immunoglobulin M (IgM), α_2 -M and α_1 -protease inhibitor (α_1 -PI) were determined by immunonephelometry. The immunoradiometric assay (IRMA) was used for measurement of all proteins in BAL. This assay, previously described in detail [20], does not require concentration of BAL fluid and provides a sensitivity in the range of the nanogram per millilitre. The specificity of this assay has also been reported previously [20]. Moreover, for measurements of BAL proteins with IRMA, the intra-assay and inter-assay coefficients of variation were, respectively, <3.5% and <4.2% for all proteins. In order to minimize the effect both of individual differences in serum protein concentration and variable dilution of BAL, the results were expressed in terms of coefficient of excretion relative to albumin (RCE):

$$\frac{[\text{Protein}] \text{ BAL}}{[\text{Protein}] \text{ Serum}} \bigg/ \frac{[\text{Albumin}] \text{ BAL}}{[\text{Albumin}] \text{ Serum}}$$

Assessment of disease duration

The duration of disease was approached using both a clinical estimate and the radiographic stage. The duration of disease was estimated from the time elapsed between the onset of symptoms or the first abnormal chest radiograph and the baseline assessment in our institution [26]. As shown in table 1, acute symptoms (erythema nodosum $n=5$, uveitis $n=1$) marked the beginning in six patients. Extrathoracic lymphadenopathies ($n=9$) and cutaneous lesions ($n=2$) were the first evidence of the disease in 11 patients. Eleven patients presented with more progressive symptoms, including respiratory complaints (dyspnoea and/or cough, $n=8$) and chronic fatigue/fever ($n=3$). Three patients were symptomless, and the first abnormal chest roentgenogram was used to estimate the duration of the disease.

In order to assess the influence of disease duration on the predictive value of BAL indices, the clinical estimate of disease duration was used as a categorical variable, and the patients were separated into three groups as follows: disease duration ≤ 6 months (sarcoidosis of recent origin, $n=14$); disease duration >6 months and ≤ 24 months (sarcoidosis of intermediate duration, $n=8$); and disease duration >24 months (longstanding sarcoidosis, $n=9$). We also conducted an analysis in a restricted

Table 1. — Presenting symptoms

Acute onset	6
Erythema nodosum	5
Uveitis	1
Clinically evident onset	11
Extrathoracic lymphadenopathies	9
Cutaneous lesions	2
Progressive onset	11
Dyspnoea and/or cough	8
Chronic fatigue	3
Asymptomatic	3

group of patients with sarcoidosis of recent origin, as defined by the presence of clinically evident symptoms (erythema nodosum, $n=4$; uveitis, $n=1$; and extrathoracic lymphadenopathies, $n=5$) for less than 6 months. Finally, the patients with radiological features of fibrotic lung distortion (Stage 4) ($n=7$) were considered as having reasonable evidence of longstanding disease [27], and were compared to patients with Stage 1, 2 and 3.

Analysis of results

Differences between patient groups were assessed using nonparametric test for unpaired samples. The comparison of initial and follow-up results of pulmonary function tests was made using nonparametric test for paired samples. The prognostic value of BAL parameters was evaluated by correlating the percentage changes in pulmonary function tests to the initial value of the BAL parameter (Spearman's rank correlation coefficient (r_s)). Values of $p \leq 0.05$ were considered significant.

Results

The study group included 14 women and 17 men, aged 26–70 yrs (median 40 yrs). Seven patients had a radiologic Stage 1; 10 Stage 2; 7 Stage 3, and 7 Stage 4. The baseline and follow-up functional features of the patients as a single group are presented in table 2. When considering the 31 patients as one group, lung function tests at the end of the follow-up period were not significantly different as compared to initial assessment, with the exception of TLCO which was significantly decreased (mean $\pm$ SD % change from initial value, $-6 \pm 10\%$; $p < 0.05$).

BAL cells and soluble components at the time of baseline assessment are shown in table 3. The total cell count, percentage of lymphocytes, T4/T8 ratio, and the α_2 -M, IgA, IgG and IgM levels were significantly

Table 2. — Results of lung function tests at baseline and follow-up assessment

	Baseline	Follow-up	$\geq 10\%$ decrease†
FVC % pred	96 $\pm$ 21	95 $\pm$ 19	4
$n < 80\%$ pred	7	7	
FEV ₁ % pred	84 $\pm$ 25	83 $\pm$ 25	7
$n < 80\%$ pred	12	11	
TLC % pred	95 $\pm$ 94	94 $\pm$ 20	6
$n < 80\%$ pred	6	4	
TLCO % pred	91 $\pm$ 18	84 $\pm$ 18*	13
$n < 80\%$ pred	8	13	
Kco % pred	92 $\pm$ 13	90 $\pm$ 13	6
$n < 80\%$ pred	6	9	

Percentage predicted data are presented as mean $\pm$ SD. †: number of patients with $\geq 10\%$ decrease at follow-up as compared to initial value; *: $p < 0.05$ when compared to initial value (nonparametric test for paired samples). FVC: forced vital capacity; FEV₁: forced expiratory volume in one second; TLC: total lung capacity; TLCO: single-breath capacity of diffusion for carbon monoxide; Kco: coefficient of diffusion for carbon monoxide (*i.e.* TLCO corrected for alveolar volume).

Table 3. — BAL data of the sarcoidosis patients at "baseline" assessment as compared to normal individuals

	Normals n=16	Sarcoidosis patients n=31
Total cell count $\times 10^4$ cells·ml ⁻¹	16.4±5.9	28.1±16.9**
Lymphocytes % total cells	9.0±7.7	20.1±11.7**
Neutrophils % total cells	0.6±0.8	0.5±0.8
T4 cells % lymphocytes	54±15	72±12**
T8 cells % lymphocytes	29±13	18±11**
T4/T8 ratio	2.5±1.9	6.1±5.5*
α_1 -PI RCE	1.12±0.57	1.61±1.39
α_2 -M RCE	0.04±0.03	0.58±0.67**
IgA RCE	1.15±0.50	1.63±0.90*
IgG RCE	0.70±0.36	1.53±0.80**
IgM RCE	0.07±0.06	0.40±0.55*

Data are presented as mean±SD. *, **: $p \leq 0.05$, 0.01 compared to normals. RCE: relative coefficient of excretion (see text for definition); α_1 -PI: α_1 -proteinase inhibitor; α_2 -M: α_2 -macroglobulin; BAL: bronchoalveolar lavage; Ig: immunoglobulin.

increased in the sarcoidosis patients, when compared to 16 healthy nonsmoking control subjects, as reported previously [20, 21].

When analysing all patients together, we found no correlation between the changes in lung volumes at the end of the follow-up period and initial BAL parameters. By contrast, we observed weak but significant correlations

between the changes in diffusion indices (TLCO or Kco) and some of the initial BAL parameters. The changes in TLCO correlated inversely with the initial IgA RCE ($r_s = -0.45$; $p < 0.05$) and IgG RCE ($r_s = -0.43$; $p < 0.05$), whilst the changes in Kco correlated inversely with the initial α_1 -PI RCE ($r_s = -0.55$; $p < 0.01$) and tended to correlate positively with the initial BAL T4/T8 ratio ($r_s = 0.33$; $p = 0.05$).

None of the BAL parameters was predictive for the evolution of the radiologic profusion score. The BAL, lung function and radiographic data of the patients separated according to disease duration and radiographic staging are detailed in table 4. Significant decreases in FVC, FEV₁, TLC and TLCO of patients with radiographic Stage 4 were observed, when compared with patients with radiographic Stage 1, 2 and 3. No significant difference was observed between the different groups of patients for BAL cellular and protein data. When the group of six patients who experienced a complete remission was analysed separately, no significant correlation was observed between BAL parameters and lung function tests.

We further assessed these correlations while separating the patients into three groups according to their estimated disease duration at the time of the BAL procedure (≤ 6 months, sarcoidosis of recent origin; 7–24 months, sarcoidosis of intermediate duration; and > 24 months, longstanding sarcoidosis) (table 5). Among the different BAL, functional and radiographic data tested, only the following correlations were observed: firstly, the

Table 4. — Radiologic profusion score, functional and BAL parameters at "baseline" evaluation according to the clinical estimate of disease duration and the radiographic stage

	Clinical estimate of disease duration [†]			Radiographic stage [#]	
	≤ 6 months n=14	7–24 months n=8	> 24 months n=9	Stage 1–3 n=24	Stage 4 n=7
Radiologic profusion score	3.5±2.1	5.5±3.0	5.1±3.2	3.9±2.5	6.6±2.4
Functional parameters					
FVC % pred	100±17	100±23	84±22	101±17	78±23*
FEV ₁ % pred	90±25	92±18	67±22	92±19	55±20*
TLC % pred	96±12	101±23	87±17	100±16	79±11*
TLco % pred	98±10	85±18	84±23	95±16	76±18*
Kco % pred	95±11	83±15	94±13	92±14	90±13
BAL parameters					
Total cell count $\times 10^4$ cells·ml ⁻¹	25.2±19.1	26.7±14.5	33.8±15.8	25.9±16.5	35.6±17.6
Lymphocytes % total cells	20.2±12.4	23.5±10.3	17.1±10.1	19.9±11.9	21.0±11.5
Neutrophils % total cells	0.8±1.0	0.2±0.3	0.2±0.3	0.4±0.8	0.6±0.9
T4 cells % lymphocytes	71±19	72±16	68±8	72±14	65±20
T8 cells % lymphocytes	15±10	18±14	23±8	17±11	20±10
T4/T8 ratio	7.7±7.7	5.7±3.1	3.3±1.5	6.5±6.1	3.9±2.9
α_1 -PI RCE	1.92±1.54	1.51±1.50	1.21±1.02	1.65±1.51	1.48±0.95
α_2 -M RCE	0.34±0.29	1.11±0.96	0.47±0.59	0.56±0.71	0.65±0.57
IgA RCE	1.59±0.98	2.04±0.79	1.54±0.74	1.69±0.91	1.70±0.77
IgG RCE	1.56±1.02	1.78±0.49	1.25±0.92	1.60±0.79	1.29±1.18
IgM RCE	0.33±0.32	0.46±0.54	0.45±0.83	0.33±0.41	0.64±0.88

[†]: the clinical estimate of disease duration was defined as the time elapsed between the onset of symptoms or the first abnormal chest radiograph and initial BAL procedure; [#]: chest radiographs were classified as Stage 1 (hilar lymphadenopathy alone), Stage 2 (parenchymal infiltrates without lymphadenopathy), Stage 3 (parenchymal infiltrates with lymphadenopathy), and Stage 4 (pulmonary fibrosis including hilar retraction, atelectasia and bullae). *: $p < 0.05$ Stage 4 vs 1–3, using unpaired Student's t-test. For abbreviations see legends to tables 2 and 3.

Table 5. — Correlations between immunoglobulin and antiprotease levels in "baseline" BAL and changes in diffusion indices according to the clinical estimate of disease duration and the radiographic stage

Correlations*	Clinical estimate of disease duration [†]						Radiographic stage [#]			
	≤6 months n=14		7–24 months n=8		>24 months n=9		Stage 1–3 n=24		Stage 4 n=7	
	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p
Initial IgA RCE vs										
Change in TLCO	-0.92	0.0008	NS		NS		-0.49	0.02	NS	
Change in KCO	NS		NS		NS		NS		NS	
Initial IgG RCE vs										
Change in TLCO	-0.75	0.006	-0.88	0.01	NS		-0.58	0.004	NS	
Change in KCO	NS		NS		NS		NS		NS	
Initial α ₁ -PI RCE vs										
Change in TLCO	NS		NS		NS		NS		NS	
Change in KCO	NS		-0.80	0.03	-0.76	0.02	-0.52	0.02	-0.85	0.01
Initial α ₂ -M RCE vs										
Change in TLCO	NS		NS		NS		NS		NS	
Change in KCO	NS		NS		NS		NS		-0.88	0.02
Initial BAL T4/T8 ratio vs										
Change in TLCO	NS		NS		NS		NS		NS	
Change in KCO	NS		NS		0.74	0.03	NS		0.78	0.05

[†], [#]: see legend to table 4 for definitions. *: correlations between variables were assessed using the Spearman's rank coefficients (r_s). NS: p>0.05 nonsignificant. Changes in TLCO and KCO during the follow-up period are evaluated as % change from initial values. RCE: relative coefficient of excretion. For further abbreviations see legends to tables 2 and 3.

Fig. 1. — Correlations between the level of IgA and IgG (expressed as RCE) in initial BAL and the change in TLCO during the follow-up period (expressed as the % change from initial value) in patients with pulmonary sarcoidosis of recent origin either defined by: a) an estimated disease duration <6 months (*i.e.* the time elapsed since the first symptom or the first abnormal chest radiograph) (n=14); or b) the presence of clinically evident symptoms (*i.e.* erythema nodosum, uveitis or extrathoracic lymphadenopathies) <6 months (n=10). TLCO: diffusion capacity for carbon monoxide; RCE: relative coefficient of excretion; IgA and IgG: immunoglobulins A and G; BAL: bronchoalveolar lavage.

Fig. 2. — Correlations between the level of α_1 -PI and α_2 -M (expressed as RCE) in initial BAL and the change in Kco during the follow-up period (expressed as the % change from initial value) in patients with longstanding pulmonary sarcoidosis either defined by: a) an estimated disease duration >24 months (*i.e.* the time elapsed since the first symptom or the first abnormal chest radiograph) ($n=9$); or the presence of radiographic Stage 4 (*i.e.* radiographic evidence of fibrotic lung distortion) ($n=7$). Kco: diffusion capacity for carbon monoxide corrected for alveolar volume; RCE: relative coefficient of excretion; α_1 -PI: α_1 -proteinase inhibitor; α_2 -M: α_2 -macroglobulin; BAL: bronchoalveolar lavage.

initial IgA RCE correlated with the changes in TLCO ($r_s = -0.92$; $p=0.0008$) (fig. 1) in patients with sarcoidosis of recent origin, but not in the other groups. The initial IgG RCE correlated with the changes in TLCO in patients with sarcoidosis of recent ($r_s = -0.75$; $p=0.006$) (fig. 1) and intermediate ($r_s = -0.88$; $p=0.01$) duration, but not in longstanding sarcoidosis. We observed an inverse correlation between the initial α_1 -PI RCE and the changes in Kco in patients with sarcoidosis of intermediate duration ($r_s = -0.80$; $p=0.03$) and with longstanding sarcoidosis ($r_s = -0.76$; $p=0.02$) (fig. 2). The correlation was absent in sarcoidosis of recent origin. The initial BAL T4/T8 ratio correlated with the changes in Kco ($r_s = 0.74$; $p=0.03$) in patients with longstanding disease. This correlation was not significant in patients with intermediate disease duration ($r_s = 0.59$; $p=0.08$), nor in patients with disease of recent origin. In all groups, initial BAL IgM did not correlate with any of the changes in lung function tests (data not shown).

When the analysis was restricted to patients with recent onset sarcoidosis, as defined by the presence of clinically evident symptoms (erythema nodosum $n=4$; uveitis $n=1$; and extrathoracic lymphadenopathies $n=5$) for less than 6 months, we found that initial IgA RCE and IgG RCE correlated with the changes in TLCO ($r_s =$

-0.95 ; $p=0.004$ and $r_s = -0.91$; $p=0.006$, respectively) (fig. 1), whilst BAL T4/T8 ratio and antiproteases were not predictive of the changes in diffusion indices in this subgroup of patients.

We also assessed the correlations in patients with radiological evidence of longstanding disease (Stage 4) ($n=7$) as opposed to patients with Stage 1, 2 and 3 considered together (table 5). The changes in TLCO correlated with the initial IgA and IgG RCE ($r_s = -0.49$; $p=0.02$ and $r_s = -0.58$; $p=0.004$, respectively) in patients with radiologic Stage 1, 2 and 3, but not in patients with Stage 4. The α_1 -PI RCE correlated inversely with the changes in Kco in Stage 4 patients ($r_s = -0.85$; $p=0.01$) (fig. 2) and this correlation was less striking in Stages 1, 2 and 3 ($r_s = -0.52$; $p=0.02$). The α_2 -M RCE also correlated negatively with the changes in Kco in Stage 4 patients ($r_s = -0.88$; $p=0.02$) (fig. 2), but not in Stages 1, 2 and 3. BAL T4/T8 ratio correlated positively with the changes in Kco in patients with a radiographic Stage 4 ($r_s = 0.78$; $p=0.05$).

Discussion

As the natural course of pulmonary sarcoidosis is unpredictable, the main problem for the clinician is to

identify those patients in whom lung function will deteriorate. Moreover, as recently emphasized by SHARMA [28], there are no well-defined criteria to start steroid treatment in patients with pulmonary sarcoidosis. In this regard, there has been considerable disagreement concerning the prognostic value of BAL cell counts [4–14]. The aim of this study was to determine whether soluble components (immunoglobulins and antiproteases) in BAL fluid could help to predict the functional evolution of patients with untreated pulmonary sarcoidosis. Considering that the disparity between studies on BAL in sarcoidosis could be related to differences in patients selection, we investigated a rather homogeneous group of patients: white, nonsmokers and untreated. However, the study population included not only newly diagnosed patients but also subjects in whom the diagnosis of sarcoidosis was previously established. This explains the relatively small proportion of patients with recent disease compared to previous studies.

We found that high BAL IgA and IgG levels at baseline assessment, expressed as a relative coefficient of excretion of albumin (RCE), correlated with subsequent changes in TLCO during the natural course of pulmonary sarcoidosis, whilst α_1 -PI RCE correlated with changes in Kco. Further analysis showed that the correlation between immunoglobulin RCE and the changes in TLCO held only for the patients with sarcoidosis of recent origin (and intermediate duration for IgG). By contrast, α_1 -PI RCE correlated with the changes in Kco predominantly in patients with longstanding disease. In sarcoidosis, there is not a unique method to distinguish between recent onset and longstanding sarcoidosis based on the estimated disease duration [9, 12, 16, 29]. We therefore separated our patients into three groups according to the estimated disease duration (<6 months; 6–24 months; and >24 months) since: 1) patients with symptoms for less than 6 months have been considered as having recent onset sarcoidosis in previous studies [9]; and 2) 24 months has been considered as the beginning of the chronic phase of the disease [9, 12, 30]. The analysis restricted to the group of patients in whom the onset of disease was based on the evidence of clinical symptoms for less than 6 months confirmed the correlations between IgA and IgG and the changes in TLCO. Also, since the presence of fibrotic lung lesions (Stage 4) on chest radiographs can be considered as an objective evidence of longstanding sarcoidosis, we separately analysed the patients with radiologic Stage 4 as opposed to those having Stage 1, 2 and 3. The comparison of the two radiologic groups largely supported the results of our analysis based on the clinical estimate of disease duration.

High IgG/albumin ratio and increased numbers of IgG secreting cells has been documented in BAL fluid from sarcoidosis patients, suggesting that immunoglobulins are produced locally [21, 31–34]. VALEYRE *et al.* [29] found a negative correlation between BAL IgG/albumin but not IgA/albumin ratio and TLCO corrected for alveolar volume in patients with sarcoidosis of recent origin (radiographic Stages 1, 2 and 3, mean disease duration of 12.5 months). We recently reported in a cross-

sectional study that BAL IgA and IgG RCE correlated inversely with diffusion indices [19]. The absence of correlation between BAL IgA and TLCO in the study by VALEYRE *et al.* [29] could be explained, at least in part, by the fact that these authors did not correct their BAL protein values for the corresponding serum values. It has been demonstrated that only B-lymphocytes present in lung interstitium and not cells present at the epithelial surface actively synthesize immunoglobulins [35]. This suggests that BAL immunoglobulin levels reflect the degree of interstitial infiltration by immunocompetent cells and/or granuloma, which could in turn contribute directly or indirectly to the impairment of TLCO.

In our study, we observed that high levels of IgA and IgG were associated with more pronounced decline in TLCO during the follow-up period, but only in patients with sarcoidosis of recent origin. This indicates that immune process could be predominantly associated with TLCO impairment in early stages of the disease. However, it remains unclear whether BAL immunoglobulins represent only markers of immunological events or play a direct role in the pathogenesis of alveolocapillary derangement [31–33]. Circulating immune complexes have been described in acute sarcoidosis [1], and it has recently been suggested that immunoglobulins, in particular IgA and IgG, could directly interact with alveolar macrophages [36].

Initial α_1 -PI RCE correlated with a decline in Kco in patients with longstanding sarcoidosis, as defined both by the clinical disease duration and radiologic Stage 4. This was also true for α_2 -M RCE but only in patients with Stage 4. It is unlikely that α_2 -M RCE simply reflects increased permeability of the respiratory membrane, since the RCE for IgM, which has a molecular weight similar to that of α_2 -M, did not correlate with diffusion indices. As recently reviewed [37], antiproteases (α_2 -M and α_1 -PI) can inhibit, at least in part, the proteolytic activity of enzymes released by neutrophils and macrophages, which are believed to play a key role in the pathogenesis of lung tissue injury potentially leading to interstitial fibrosis. The immunoassay that we used does not allow a distinction to be made between native and complexed antiproteases, nor estimation of the actual antiprotease activity. However, it is reasonable to assume that antiprotease levels are indirect markers of the proteolytic burden generated by inflammatory cells present in the lung interstitium and alveolar space. Thus, we recently reported that BAL fluid from sarcoidosis patients contains enzymatic activities, such as plasminogen activator and a macrophage-derived metaloprotease, which are not present in BAL from normal individuals [38]. Although the role of these proteolytic activities remains to be clarified, they are likely to interact with lung matrix components.

We have no explanation for the observation that, immunoglobulins correlate predominantly with TLCO changes whilst antiproteases correlate with the changes in Kco. Therefore, the potential pathophysiological relevance of correlations with Kco changes for some baseline BAL components and with TLCO for others remains uncertain.

As in previous studies [6, 10–16], we found that the initial BAL lymphocyte count had no prognostic significance. However, in patients with longstanding sarcoidosis, a low initial T4/T8 ratio was associated with more pronounced deterioration of Kco during the follow-up period. In contrast, the BAL T4/T8 ratio had no predictive value in patients with sarcoidosis of recent origin. These results are close to those of VERSTRAETEN *et al.* [15], who reported that patients with high BAL T4/T8 ratio show significantly greater improvement in their chest roentgenograms and TLCO after 22–36 months compared to patients with low BAL T4/T8. However, these authors did not provide precise information about the duration of disease at the time of the initial assessment.

The clinical relevance of the correlations between immunoglobulins, antiproteases, T4/T8 ratio and changes in diffusion indices during the natural course of pulmonary sarcoidosis remains largely uncertain. In our study, BAL parameters were predictive only for the changes in diffusion but not for the evolution of other lung function tests or for radiographic changes. This, however, is consistent with the recent data by VERSTRAETEN *et al.* [15], who found that BAL T4/T8 ratio correlated with the changes in TLCO but not with other functional tests. Only a minority of our patients showed marked changes of diffusion indices at the end of the follow-up period. Furthermore, there was considerable overlap between sarcoidosis patients and normal controls with respect to immunoglobulins, antiproteases and T4/T8 ratio in BAL. Although it is difficult to apply the correlations found in this study to determine the prognosis of a given patient, the most pronounced decline in diffusion indices during the follow-up period were clearly identified by the above-mentioned BAL indices (figs 1 and 2).

In conclusion, we observed that, in patients with sarcoidosis of recent origin, high IgA and IgG RCE in the initial BAL was associated with deterioration of TLco during the follow-up period. By contrast, in patients with longstanding sarcoidosis, high initial α_1 -PI RCE and low T4/T8 ratio were associated with deterioration of Kco. From a pathophysiological point of view, we conclude that BAL immunoglobulin, antiprotease levels and T4/T8 ratios could be more reliable than crude cell counts in assessing immune and inflammatory processes associated with changes of diffusion indices during the natural course of the disease. Furthermore, our observations could suggest that impairment in diffusion indices is predominantly related to the infiltration of bronchoalveolar structures by immunocompetent cells (as assessed by BAL immunoglobulins RCE) in recent sarcoidosis and to inflammatory processes (as assessed by BAL antiproteases RCE) likely to contribute to interstitial damage in longstanding sarcoidosis.

However, since no significant difference in BAL immunoglobulins and antiproteases levels was observed between patients with recent and longstanding disease, definitive conclusions with respect to clinical usefulness of the predictive value of BAL immunoglobulins and

antiproteases in sarcoidosis require further assessment in studies including larger number of patients with longer follow-up. Nevertheless, our data demonstrate that disease duration is of considerable importance in assessing the value of prognostic indicators, since different pathophysiological mechanisms may be predominant at different stages of pulmonary sarcoidosis.

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