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Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis

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Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. M.W. Ziegenhagen, M.E. Rothe, M. Schlaak, J. Müller-Quernheim. ©ERS Journals Ltd 2003.

ABSTRACT: The aim of the present study was to determine which bronchoalveolar lavage fluid (BALF) and serological parameters reflect the severity of newly diagnosed pulmonary sarcoidosis.

Seventy-four previously untreated sarcoid patients were categorised into three groups: 10 patients with Löfgren's syndrome, 51 patients with stable disease and 13 patients with progressing disease requiring systemic steroid treatment.

Total BALF cell count, percentage of alveolar lymphocytes and lymphocyte CD4/CD8 ratio were not associated with severity of disease. Interestingly, a significant increase in percentages of BALF neutrophils (5.2±1.1%) and eosinophils (1.7±0.6%) was observed in sarcoid patients with progressing disease. Elevated percentages of these two cell types were the only BALF parameters associated with a more frequent necessity for systemic steroid therapy. This association between an elevated percentage of BALF neutrophils and the necessity for steroid treatment was observed in advanced as well as early sarcoidosis (radiological types I and II). Serum levels of soluble interleukin-2 receptor and neopterin were significantly elevated in progressing disease compared to stable disease or Löfgren's syndrome.

The present results demonstrate that increased percentages of neutrophils (>3.0%) and eosinophils (>1%) in bronchoalveolar lavage fluid from newly diagnosed pulmonary sarcoidosis is associated with a significantly higher risk of necessity for steroid therapy and may be helpful markers of progressive disease. Furthermore, of the serological parameters investigated, only serum levels of soluble interleukin-2 receptor and neopterin were associated with disease severity.

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Sarcoidosis is a systemic granulomatous disease of unknown origin. Immunological studies performed with cells obtained by bronchoalveolar lavage (BAL) have considerably improved knowledge of its immunopathogenesis [1–3]. A typical feature of pulmonary sarcoidosis is an increase in the percentage of BAL fluid (BALF) lymphocytes with an accumulation of T-helper cells in the lung, resulting in an increased BALF lymphocyte CD4/CD8 ratio.

Despite frequent use of BAL in establishing the diagnosis, no consensus has yet been achieved as to which of the cellular compartments of the BALF reflect the severity of newly diagnosed pulmonary sarcoidosis. Since the 1980s, several studies analysing the role of the percentage of BALF lymphocytes and the CD4/CD8 ratio have been performed with diverse results [4–7]. Since the late 1990s, however, there has been increasing evidence that neutrophilic alveolitis may play an important role in the outcome of sarcoidosis. In a recent study, increased release of pro-inflammatory chemokines (*e.g.* interleukin (IL)-8, a potent neutrophil chemotactic factor) by BALF cells in progressing sarcoidosis and idiopathic pulmonary fibrosis was demonstrated [8]. In the same study, the percentage of BALF neutrophils was significantly higher in progressing disease compared to stable disease. Interestingly, DRENT *et al.* [9] recently demonstrated, in a study of 26 sarcoid patients, that the number of neutrophils in the BALF distinguished between patients who experienced spontaneous remission and those having a more severe course of disease.

Several serum components are used as markers of disease activity in sarcoidosis [10]. The most commonly used markers are serum angiotensin-converting enzyme (ACE), soluble IL-2 receptor (sIL-2R) and neopterin. Although levels of these markers are closely linked to the pathogenesis of the disease, insufficient evidence is available as to which of them is suitable for assessment of the severity of sarcoidosis.

The aim of the present study was to investigate, in a large cohort of sarcoid patients (n=74), which BALF and serological parameters reflect the severity of newly diagnosed sarcoidosis and may be helpful markers of progressive disease.

Methods

Study population

A diagnosis of sarcoidosis was established in 74 patients with newly diagnosed pulmonary sarcoidosis in accordance with recently described criteria [11]. None of the patients included in this study had previously been treated with steroids; in all patients, noncaseating granulomas were identified by transbronchial and/or endobronchial biopsy. BAL and histological confirmation were performed *via* the same bronchoscopic procedure at initial presentation. The time point of bronchoscopy was regarded as the time point of diagnosis.

Microbiological analysis of BALF was negative for bacteria, mycobacteria and fungi in all patients.

The 74 sarcoid patients were allocated to three groups: 10 newly diagnosed sarcoid patients had classical Löfgren's syndrome (bihilar adenopathy, arthritic symptoms and erythema nodosum) without indication for systemic steroid therapy at the time of diagnosis or during the following 6 months (Löfgren's syndrome group); 51 patients showed no evidence of deterioration in lung function parameters at the time of diagnosis or during the following 6 months and no extrapulmonary manifestation requiring systemic steroid therapy (stable disease group); 13 patients showed clear evidence of a progressing disease (significant decline in lung function parameters) or an extrapulmonary manifestation requiring therapy at the time of diagnosis or within the following 6 months (progressing disease group). Only one of these 13 patients was treated for severe systemic disease with multiple extrapulmonary manifestations of sarcoidosis (skin, eye, liver and severe general symptoms). This patient had radiological type I sarcoidosis; histological evidence of pulmonary involvement was determined by biopsy of the bronchial mucosa. The other 12 patients were treated due to there being clear evidence of progressing pulmonary sarcoidosis. The definitions used for detection of a significant decline in lung function parameters were the same as those recently described by HUNNINGHAKE *et al.* [12]. In brief, for total lung capacity (TLC) and diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$), a 10% decline from baseline was regarded as significant. For vital capacity (VC) and forced expiratory volume in one second (FEV_1), a 15% decline from baseline was regarded as significant.

The control group consisted of 48 individuals who underwent bronchoscopy for diagnostic reasons and were retrospectively free of any infectious, inflammatory or malignant lung disease. Microbiological analysis of the BALF was negative for bacteria, mycobacteria and fungi. Transbronchial biopsy was not performed in the control population, because the chest radiograph disclosed no evidence of interstitial or other abnormalities. The control group was age-matched and had a similar ratio of smokers and nonsmokers as the sarcoidosis cohort. The BALF characteristics of the control population are summarised in table 1. An increase in total cell count and alveolar macrophage number and a decrease in the percentage of lymphocytes in smokers compared to nonsmokers were observed. The percentage of neutrophils did not differ significantly, whereas the total number of neutrophils was higher in smokers ($0.11\pm0.05\times10^4$ *versus* $0.05\pm0.01\times10^4$ cells·mL^{-1}), but this difference did not reach significance. These observations are in line with the results of a large multicentric study performed in healthy individuals [13].

Table 1. – Bronchoalveolar lavage fluid characteristics of the control population

Control	Nonsmokers	Smokers
Subjects n	39	9
Total cell count 10^4 cells·mL^{-1}	5.1±0.5	37.4±13.3#
AM %	93.9±0.7	96.9±0.6*
Lymph. %	5.2±0.7	2.1±0.5*
Neut. %	0.8±0.1	0.5±0.2
Eosin. %	0.01±0.03	0.5±0.2
AM 10^4 cell·mL^{-1}	4.8±2.9	36.6±13.2#
Lymph. 10^4 cell·mL^{-1}	0.2±0.04	0.6±0.2*
Neut. 10^4 cell·mL^{-1}	0.05±0.01	0.11±0.05
Eosin. 10^4 cell·mL^{-1}	0.0±0.0	0.2±0.1*

Data are presented as mean±SEM. AM: alveolar macrophages; Lymph.: lymphocytes; Neut.: neutrophils; Eosin.: eosinophils. *: $p<0.05$; #: $p<0.0001$ *versus* nonsmokers.

Lung function and arterial oxygen tension measurement and chest radiography

FEV_1, VC, TLC and FEV_1/VC were measured using a body plethysmograph (Master Screen Body; Jaeger, Würzburg, Germany) and $D_{L,CO}$ was determined by the single-breath method. Values are expressed as percentage of the predicted value. All pulmonary function tests were performed according to the recommendations of the European Respiratory Society [14]. Arterial oxygen tension (P_{a,O_2}) was measured at rest without supplementary oxygen.

Chest radiography was performed in posterior/anterior and lateral projection. Three different experienced readers classified the radiographs according to the radiographical types of sarcoidosis (0–IV).

Bronchoalveolar lavage and preparation of bronchoalveolar lavage fluid cells

Bronchoscopy and BAL were performed as previously described [1]. In brief, 300 mL sterile saline (0.9% NaCl) was instilled into a lingula or middle-lobe segment in 25 mL aliquots. Each aliquot was immediately aspirated and the recovered aliquots were pooled. Differential cell counts were determined using cytospin preparations of $\geqslant$400 cells (Cytospin II; Shandon Instruments, Sewickley, PA, USA). In cytospin preparations with percentages of cell populations near the established threshold values, $\geqslant$800 cells were counted. For immunoperoxidase staining [15], cells were fixed on poly-L-lysine-coated slides (Bio-Rad, Munich, Germany) and developed with a peroxidase/antiperoxidase technique using monoclonal antibodies directed against CD4, CD8, IL-2R (Ortho Diagnostic Systems, Neckargemünd, Germany) and human leukocyte antigen DR (Becton Dickinson, Heidelberg, Germany). Informed consent was obtained from all participants in this study.

Establishing the threshold of bronchoalveolar lavage fluid parameters

The large cohort of 48 controls was used to establish the threshold for the BAL differential cell counts. Since the percentages of BAL cells follow a nonparametric distribution, the 95th percentile of the control population was chosen as the threshold for the percentages of BAL lymphocytes, neutrophils and eosinophils. The 95th percentile was 11.3% for lymphocytes, 2.5% for neutrophils, 1.0% for eosinophils and 3.3 for the BAL lymphocyte CD4/CD8 ratio. For practical reasons, values of >15% lymphocytes, >3.0% neutrophils, >1.0% eosinophils and a CD4/CD8 ratio of >3.5 were regarded as elevated. These established thresholds are in accordance with the results of a large multicentric study performed in healthy individuals [13].

Serological parameters

Serum ACE, neopterin and sIL-2R levels were determined using commercially available kits, as described previously [6]. C-reactive protein (CRP) concentration was measured by an immunological method using rabbit antihuman CRP (DAKO A/S, Glostrup, Denmark) and an ELAN analyser (Eppendorf, Hamburg, Germany). The normal range was 0–0.50 mg·dL^{-1}. All normal ranges were used according to the recommendations of the manufacturers of the tests.

Statistical analysis

Data are expressed as mean±SEM. Comparisons were performed using the Mann-Whitney U-test. Differences between groups were evaluated with the Chi-squared test. Correlations between different parameters were determined using Spearman's rank correlation coefficient. A p-value of <0.05 was regarded as significant.

Results

Clinical characteristics of the study population

The clinical characteristics of the study population are summarised in table 2. The mean age of the sarcoidosis group (n=74) and that of the controls (n=48) did not differ significantly. Among the sarcoid subgroups according to disease severity, patients with Löfgren's syndrome were slightly younger than those with stable disease (33.1±2.8 *versus* 38.7±1.6 yrs; p=NS) but significantly younger than those with progressive disease (33.1±2.8 *versus* 52.7±4.2 yrs; p<0.005). Patients with stable disease were also significantly younger than those with progressive disease (p<0.005).

The percentage of smokers was 23.1 in the control group and 25.4 in the sarcoidosis group. None of the sarcoid patients with progressive disease smoked.

The CRP level in the controls (0.48±0.11 mg·dL^{-1}) was within the normal range. The CRP level in the sarcoidosis group (1.43±0.30 mg·dL^{-1}) was significantly higher (p<0.01). This difference was due to a highly significant increase in CRP level in the Löfgren syndrome subgroup (6.0±1.3 mg·dL^{-1}; p<0.0001 *versus* controls and patients with stable disease (0.73±0.15 mg·dL^{-1}); p<0.001 *versus* patients with progressing disease (0.84±0.19 mg·dL^{-1})). The CRP level in patients with stable or progressing disease did not differ significantly from that in controls. There was no correlation between CRP level and percentage of BALF neutrophils (ρ=0.26; p=NS).

The VC, TLC and $D_{L,CO}$ of the sarcoidosis group were significantly lower than in the controls (p<0.05), but still within the normal range. The FEV1/VC ratio did not differ between the sarcoidosis and control group. The lung function parameters of the Löfgren syndrome group did not differ from those of controls, whereas the VC and TLC of the patients with stable disease were significantly lower than in controls, but also still within the normal range. The VC and the $D_{L,CO}$ of the sarcoid patients with progressing disease were significantly lower than in controls and sarcoid patients with Löfgren's syndrome or stable disease (table 2). The FEV1/VC ratio did not differ significantly between the sarcoidosis subgroups.

P_{a,O_2} did not differ significantly between the control and the entire sarcoidosis groups (n=74). The P_{a,O_2} of sarcoid patients with Löfgren's syndrome or stable disease was not significantly different from that in controls, whereas the P_{a,O_2} of sarcoid patients with progressive disease was significantly lower than in controls and sarcoid patients with Löfgren's syndrome or stable disease (table 2).

Analysis of bronchoalveolar lavage fluid parameters

The percentage of BALF lymphocytes was significantly elevated in the sarcoidosis group compared to controls (15.9±1.4 *versus* 4.6±0.6%; p<0.0001). In all sarcoidosis subgroups, the percentage of BALF lymphocytes was significantly higher than in controls, whereas no significant differences were obtained between the sarcoid subgroups (fig. 1). The CD4/CD8 ratio was significantly increased in the sarcoidosis group and in all sarcoidosis subgroups compared to controls. The highest CD4/CD8 ratio was observed in the Löfgren syndrome group (6.0±1.3) but this level was not significantly higher than that in patients with stable or progressing disease. The percentage of neutrophils was insignificantly higher in the sarcoidosis group than in controls (1.6±0.3 *versus* 0.8±0.1%). A highly significant increase in the percentage of BALF neutrophils was observed in sarcoid patients with progressing disease compared to controls (5.2±1.1 *versus* 0.8±0.1%; p=0.0005), as well as compared to patients with Löfgren's syndrome or stable disease (p<0.05 and p<0.0005, respectively). The percentage of BALF eosinophils was slightly elevated in the sarcoidosis group compared to controls (0.4±0.1 *versus*

Table 2. – Characteristics of the study population

	Controls	Sarcoid patients			
		Entire cohort	Löfgrens syndrome	Stable disease	Progressing disease
Subjects n	48	74	10	51	13
Age yrs	42.3±1.9	40.4±1.5	33.1±2.8*,¶¶	38.7±1.6¶¶	52.7±4.2*
Smokers/nonsmokers	9/39	15/59	2/8	13/38	0/13
C-reactive protein mg·dL^{-1}	0.48±0.11	1.43±0.30*	6.0±1.3#,¶¶	0.73±0.15	0.84±0.19
Lung function					
VC % pred	101.7±3.1	90.3±2.2*	100.3±3.0¶¶	93.2±2.3*,¶¶	74.3±5.8***
TLC % pred	99.7±2.7	88.2±2.1*	99.6±5.6¶	89.2±2.1*	77.4±5.1***
$D_{L,CO}$ % pred	90.9±3.7	78.8±2.6*	88.2±3.3¶¶	83.8±2.5¶¶	54.0±4.6#
FEV1/VC	82.1±1.2	80.6±1.6	84.6±1.7	80.3±1.7	79.5±5.2
P_{a,O_2} mmHg	86.1±1.1	86.5±1.5	90.2±4.4¶	89.1±1.4¶¶	72.1±2.8#
BALF					
Total cell count 10^4 cells·mL^{-1}	11.9±3.4	12.2±1.0*	13.4±2.6***	11.2±1.1*	13.1±2.2*
AM 10^4 cells·mL^{-1}	11.4±3.3	9.5±0.8*	9.8±2.0	9.3±1.0	10.2±1.9
Lymph. 10^4 cells·mL^{-1}	0.3±0.1	1.9±0.3#	2.4±0.8#	1.8±0.4#	2.1±0.5#
Neut. 10^4 cells·mL^{-1}	0.06±0.01	0.18±0.04	0.13±0.0¶	0.08±0.02¶¶	0.66±0.16#
Eosin. 10^4 cells·mL^{-1}	0.04±0.02	0.05±0.02	0.01±0.01¶	0.03±0.01¶¶	0.18±0.07#

Data are presented as mean±SEM. VC: vital capacity; TLC: total lung capacity; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; FEV1: forced expiratory volume in one second; P_{a,O_2}: arterial oxygen tension; BALF: bronchoalveolar lavage fluid; AM: alveolar macrophages; Lymph.: lymphocytes; Neut.: neutrophils; Eosin.: eosinophils; % pred: percentage of the predicted value. *: p<0.05; ***: p<0.001; #: p<0.0005 *versus* control; ¶: p<0.05; ¶¶: p<0.005 *versus* progressing disease. 1 mmHg=0.133 kPa.

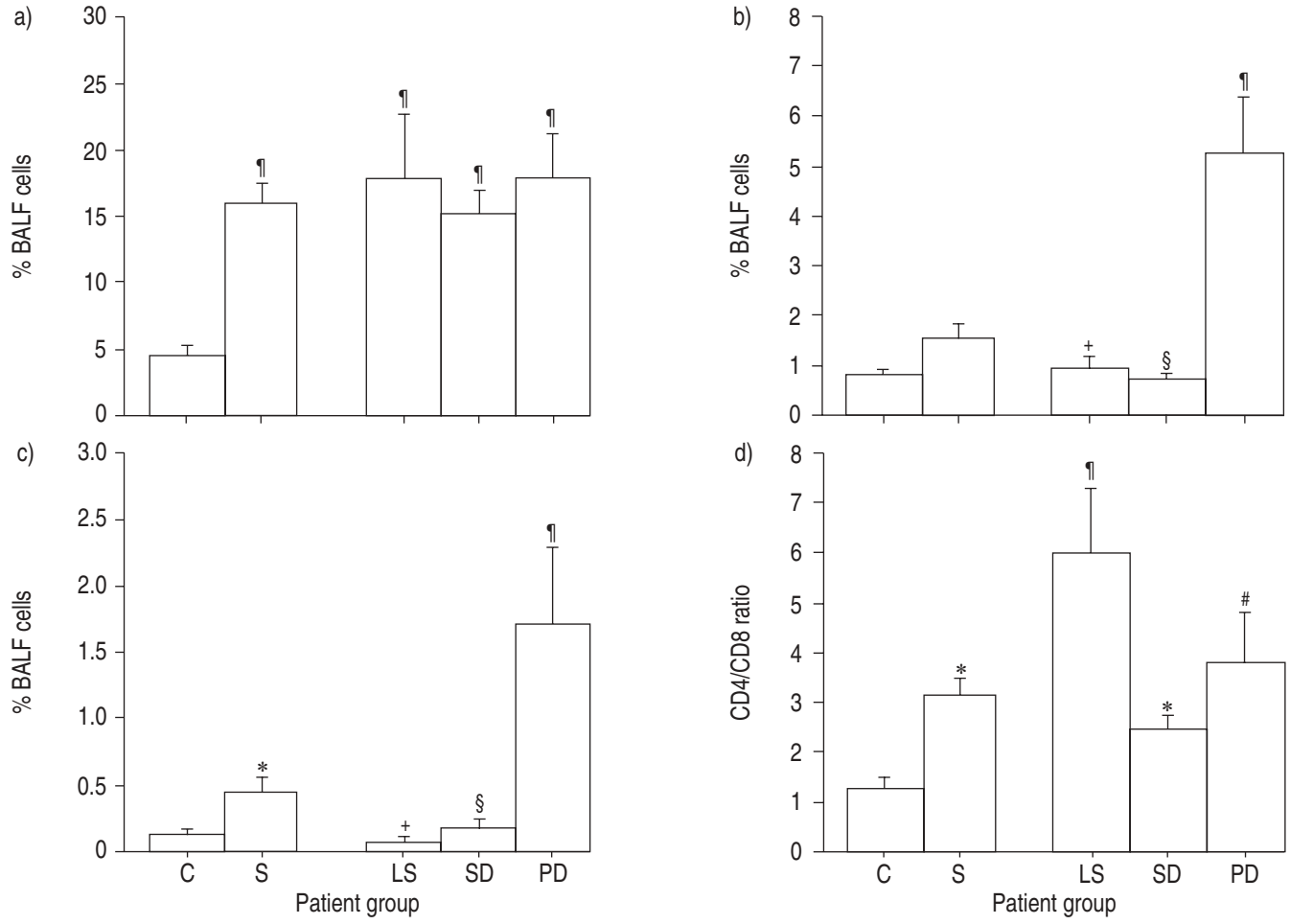

Fig. 1. – Percentages of a) lymphocytes, b) neutrophils and c) eosinophils, and d) CD4/CD8 ratio in bronchoalveolar lavage fluid (BALF) from controls (C), entire sarcoidosis cohort (S; n=74) and sarcoidosis subgroups (LS: Löfgren's syndrome; SD: stable disease; PD: progressing disease) according to disease severity. Vertical bars represent SEM. *: $p<0.05$; #: $p<0.005$; ¶: $p<0.0005$ *versus* control; +: $p<0.05$; §: $p<0.0005$ *versus* progressing disease.

0.1±0.1%; $p<0.05$). A highly significant increase in the percentage of BALF eosinophils was observed in sarcoid patients with progressing disease compared to controls (1.7±0.5 *versus* 0.1±0.1%; $p<0.0001$), as well as compared to patients with Löfgren's syndrome or stable disease ($p<0.01$ and $p<0.0005$, respectively).

Similar results were obtained when analysing the absolute numbers of BALF cells (data and significance levels are given in table 2). A highly significant positive correlation was observed between the percentage of BALF neutrophils and eosinophils ($p<0.0001$; $\rho=0.54$) and between the absolute number of both cell types ($p<0.0001$; $\rho=0.55$) in the sarcoidosis group (n=74). This positive correlation was even more pronounced as regards the subgroup of sarcoid patients with progressing disease (n=13) (percentage of neutrophils and eosinophils: $p<0.005$; $\rho=0.93$; absolute numbers of neutrophils and eosinophils: $p<0.05$; $\rho=0.69$).

Influence of smoking habits upon bronchoalveolar lavage fluid parameters in sarcoidosis

The total cell count was significantly lower in nonsmokers (n=59) than in smokers (n=15) ($10.2\pm0.9\times10^4$ *versus* $17.9\pm2.0\times10^4$ cells·mL^{-1}; $p<0.0005$). The percentage of lymphocytes was significantly higher in nonsmokers than in smokers (17.9±2.0 *versus* 8.6±2.1%; $p<0.005$), whereas no differences were obtained comparing absolute numbers of lymphocytes ($p=0.5$). No significant differences between nonsmokers and smokers were obtained comparing CD4/CD8 ratio ($p=0.9$), neutrophil percentage ($p=0.17$) or absolute numbers of neutrophils ($p=0.9$). The percentage (0.55±0.16 *versus* 0.02±0.20; $p<0.05$) as well as the absolute number of eosinophils ($0.065\pm0.021\times10^4$ *versus* $0.002\pm0.002\times10^4$ cells·mL^{-1}; $p<0.05$) was slightly elevated in nonsmokers compared to smokers.

Percentage of bronchoalveolar lavage fluid neutrophils and radiographical type of sarcoidosis

The relationship between percentage of BALF neutrophils, radiographical type of sarcoidosis and necessity for systemic steroid therapy is demonstrated in figure 2. Only one of the 61 sarcoid patients without indication for therapy had an elevated percentage of BALF neutrophils (>3.0%). In contrast, nine of 13 patients with an indication for systemic steroid therapy had an elevated percentage of BALF neutrophils. An association between elevated percentages of neutrophils in BALF and necessity for systemic steroid therapy was observed in not only patients with advanced sarcoidosis (radiological types III and IV) but also sarcoid patients with earlier stages (radiological types I and II).

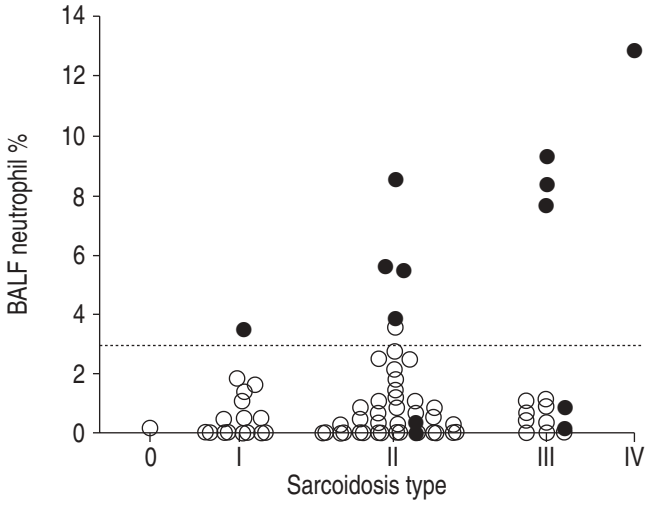

Fig. 2. – Relationship between radiographic type of sarcoidosis, percentage of bronchoalveolar lavage fluid (BALF) neutrophils and necessity for systemic steroid therapy (○: no therapy (n=61); ●: therapy (n=13)). ·····: upper limit of normal range.

Which bronchoalveolar lavage fluid parameters indicate a higher risk of necessity for steroid therapy?

Twenty-one sarcoid patients had an elevated percentage of BALF lymphocytes (>15%). Six of the 21 patients with BALF lymphocytosis and seven of 53 without BALF lymphocytosis needed corticosteroid treatment (p=NS). Sixteen patients showed an elevated CD4/CD8 ratio (>3.5). Three of the 16 patients with and 10 of 58 patients without an elevated CD4/CD8 ratio required treatment (p=NS). Ten patients showed an increased percentage of BALF neutrophils (>3.0%). Nine of the 10 patients with and four of 64 patients without an increased percentage of BALF neutrophils exhibited clear-cut indications for steroid treatment ($p<0.0001$) (fig. 2). Nine patients had an increased percentage of BALF eosinophils (>1.0%). Six of the nine patients with and seven of 65 patients without an increased percentage of BALF eosinophils needed treatment with steroids ($p<0.01$).

Analysis of serological parameters

Mean levels of serum ACE, sIL-2R and neopterin were slightly elevated in the sarcoidosis group (fig. 3). Serum ACE level was significantly lower in the Löfgren group, whereas there was no difference in serum ACE level between sarcoid patients with stable or progressing disease. The serum levels of sIL-2R and neopterin were significantly higher in sarcoid patients with progressive disease than in patients with stable disease or Löfgren′s syndrome.

Discussion

The aim of the present study was to evaluate which of the most commonly used BALF and serological parameters reflect the severity of newly diagnosed sarcoidosis. In a large cohort of 74 previously untreated patients, it was possible to demonstrate that the percentage of neutrophils and eosinophils in BALF is significantly elevated in sarcoid patients requiring systemic steroid therapy. Interestingly, the association between an elevated percentage of BALF neutrophils and necessity for steroid therapy was observed in not only patients with advanced sarcoidosis (radiological types III and IV) but also patients with earlier stages (radiological types I and II). Although the percentage of BALF lymphocytes and the BALF lymphocyte CD4/CD8 ratio may be helpful in establishing a diagnosis of sarcoidosis [11], they did not reflect the severity of the disease. The present results demonstrate that an increased percentage of BAL neutrophils (>3.0%) and eosinophils (>1.0%) reflect the severity of sarcoidosis and may be helpful markers of progressive disease. Furthermore, of the serological parameters investigated, only sIL-2R and neopterin levels reflected disease severity, whereas the most commonly used parameter, serum ACE level, was not helpful.

The utility of BALF cellular analysis in predicting the course and prognosis of sarcoidosis is still controversial. Since the 1980s, most researchers have focused their interest upon the intensity of lymphocytic alveolitis and the CD4/CD8 ratio with diverse results. Some authors found that a high percentage of BALF lymphocytes predicts functional deterioration [16], whereas others demonstrated that a high

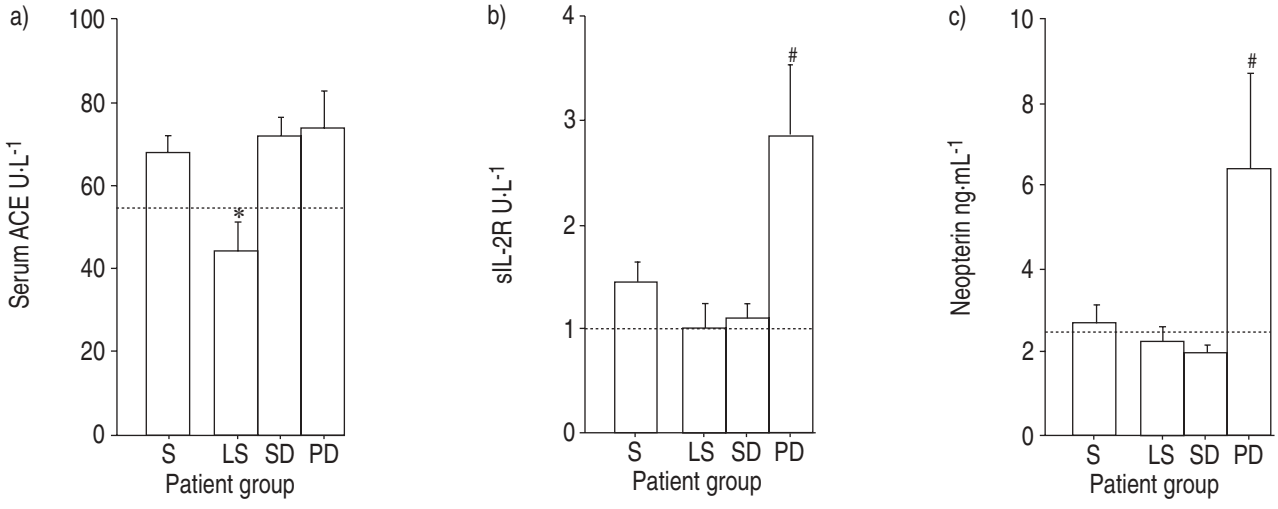

Fig. 3. – Serum levels of the investigated serological markers of disease activity in the sarcoid subgroups. a) Serum angiotensin-converting enzyme (ACE), b) soluble interleukin-2 receptor (sIL-2R) and c) neopterin (S: entire sarcoidosis cohort (n=74); LS: Löfgren′s syndrome; SD: stable disease; PD: progressing disease). Vertical bars represent SEM. ·····: upper limit of normal range. *: $p<0.05$ *versus* stable disease and progressing disease; #: $p<0.05$ *versus* Löfgren′s syndrome and stable disease.

lymphocyte count and CD4/CD8 ratio may herald alleviation of the disease [5, 17]. In other studies, neither the percentage of BALF lymphocytes nor the CD4/CD8 ratio was of predictive value [4, 6]. In the present study, neither the percentage nor the absolute number of lymphocytes or CD4/CD8 ratio reflected disease severity or indicated higher risk of necessity of steroid therapy.

The instilled volume used for BAL is crucial because it influences the differential cell count. It is known that the number of neutrophils and epithelial cells and amount of debris of bronchial origin obtained decrease with higher volume [18]. Furthermore, it has been shown that abnormalities in the cell population of the BALF due to interstitial lung diseases are more striking when a larger volume is used [19]. Therefore, 300 mL instilled volume was used for BAL to reduce possible contamination from bronchial fluid and to ensure that the observed alterations in differential cell count reflect inflammatory processes in the alveoli.

When considering the percentage of BALF neutrophils, the role of cigarette smoking should be addressed. In the present study population, there were no significant differences between the percentages of BALF neutrophils of nonsmokers and smokers in controls or in the sarcoidosis population. The results in the control population are in line with observations from a large multicentric study performed in healthy individuals [13]. Furthermore, none of the sarcoid patients who had to be treated with steroids were smokers. The main findings of the study, *i.e.* a significant increase in the percentage of BALF neutrophils only in progressing disease and no significant differences in the percentage of BALF lymphocytes or CD4/CD8 ratio between sarcoid subgroups, were similar when comparing sarcoid subgroup smokers to control smokers and sarcoid subgroup nonsmokers to control nonsmokers (data not shown). Thus, the elevation of BALF neutrophils in sarcoid patients with progressing disease is not due to effects of cigarette smoking.

Analysis of BALF cytokine levels has considerably improved knowledge regarding the immunopathogenesis of sarcoidosis [2, 3]. CAR *et al.* [20] demonstrated significantly elevated levels of IL-8 in the BALF of patients with sarcoidosis and idiopathic pulmonary fibrosis. In a recent study, it was shown that cultured BALF cells from sarcoid and idiopathic pulmonary fibrosis patients with progressive disease release significantly more pro-inflammatory chemokines, *e.g.* IL-8, than BALF cells from patients with stable disease [8]. In the same study, the percentage of BALF neutrophils was significantly higher in progressing disease than in stable disease. Since IL-8 is a potent neutrophil chemotactic factor, the observed increase in levels of this pro-inflammatory chemokine may explain the observed accumulation of BALF neutrophils in progressing disease. Thus, the neutrophilic alveolitis seen in sarcoidosis seems to reflect an important ongoing inflammatory process.

Interestingly, DRENT *et al.* [9] recently demonstrated that the number of neutrophils in BALF could be used to differentiate between patients who underwent spontaneous remission and those exhibiting a more severe course of disease. These authors pointed out that their results should be interpreted with caution due to the small size of their sarcoid cohort (n=26). The fact that the present study yielded similar results in an almost three-fold bigger population further supports the importance of neutrophilic alveolitis as a pivotal indicator of disease severity in sarcoidosis. Thus, cytokine analysis in BALF [8, 20], the results of DRENT *et al.* [9] and the data presented herein strongly corroborate, and to some extent explain, the earlier findings from the 1980s of ROTH *et al.* [21], who described a significantly higher proportion of neutrophils in 14 patients with advanced sarcoidosis, and LIN *et al.* [22], who suggested that BALF neutrophils can be used to assess the activity of the disease. In the study of ROTH *et al.* [21], patients with radiological type III sarcoidosis were classified as having advanced sarcoidosis. Although the percentage of BALF neutrophils tended to increase with radiological type in the present cohort, an association between an elevated percentage of neutrophils in BALF and the necessity for systemic steroid therapy in sarcoid patients with earlier stages (radiological types I and II) was also observed (fig. 2).

Concerning the serological parameters of disease activity investigated, only sIL-2R and neopterin levels reflected the severity of sarcoidosis. Both were significantly elevated in progressing disease compared to stable disease (fig. 3). Serum ACE level, which is thought to reflect the granuloma burden, did not differ significantly between patients with stable or progressing sarcoidosis. Interestingly, serum ACE levels were significantly lower in sarcoid patients with Löfgren′s syndrome. In this as well as various other studies [6, 23, 24], serum ACE levels were of poor predictive value in sarcoidosis. This may be due to the extraordinary high variability in serum ACE concentration in health, which is caused by numerous factors. The most important factor, responsible for ~25% of the variation, is a deletion/insertion polymorphism in intron 16 of the ACE gene [25, 26]. The question whether genotype-corrected normal values might be able to increase the role of serum ACE levels as a marker of disease activity in sarcoidosis remains to be elucidated by further studies. T-cell activation and activation of the monocyte/macrophage system play an important role in the immunopathogenesis of sarcoidosis [3]. T-cell activation can be estimated by measurement of sIL-2R levels and activation of the monocyte/macrophage system by assessment of neopterin levels. The present data support the usefulness of these two serological parameters in assessing sarcoidosis severity.

Another interesting finding of the present study concerns serum CRP levels in sarcoidosis. A significant increase in CRP levels in sarcoid patients with Löfgren′s syndrome was observed. This increase was not due to bacterial infection since clinical signs were absent, microbiological culture of BALF was negative and there was no correlation between CRP level and percentage of BALF neutrophils. The CRP levels of patients with stable or progressing disease did not differ significantly from those of controls. CRP is an acute phase protein and elevated levels are obtained in a variety of inflammatory processes. In sarcoidosis, elevated CRP levels have recently been described in patients with constitutional symptoms [27]. Since patients with Löfgren′s syndrome are known to have the best prognosis, it is tempting to speculate that an effective acute phase response may be beneficial in overcoming the still unknown cause of sarcoidosis. Thus, high disease activity on manifestation of the disease is associated with a good prognosis, whereas low-but-persisting chronic disease activity, *e.g.* reflected by an increase in percentages of BALF neutrophils and eosinophils, seems to be associated with poor outcome.

An increase in the percentage of neutrophils (>3.0%) and eosinophils (>1.0%) in BALF is certainly not sufficient to establish a diagnosis of sarcoidosis, because there are an abundance of interstitial lung diseases causing similar or even higher percentages of these cells in BALF. Nevertheless, the present results demonstrate that an increased percentage of BALF neutrophils and eosinophils is not a rare finding in sarcoidosis and reflects an ongoing inflammatory process, which may result in progressive loss of lung parenchyma.

In conclusion, the present study indicates that a low degree of neutrophilic and eosinophilic alveolitis reflects the severity of newly diagnosed sarcoidosis. Furthermore, elevated percentages of these two cell types were the only parameters of bronchoalveolar lavage fluid to predict a higher risk of necessity of systemic steroid therapy. Thus, neutrophils and

eosinophils may play an important role in the inflammatory process and outcome of sarcoidosis. The present results provide strong evidence that an increased percentage of these cell types in bronchoalveolar lavage fluid may be helpful markers of progressing disease in newly diagnosed pulmonary sarcoidosis. Further research to elucidate the precise role of these cells in the immunopathogenesis of sarcoidosis is necessary. Although lymphocytic alveolitis accompanied by an increased CD4/CD8 ratio is helpful in establishing a diagnosis of sarcoidosis, it does not reflect the severity of the disease. Furthermore, the present data demonstrate that serological assessment of soluble interleukin-2 receptor and neopterin levels may be helpful parameters in clinical practice for providing insight into the immunological processes of sarcoidosis which influence disease severity.

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