

Bronchoalveolar Lavage Fluid Profiles in Sarcoidosis, Tuberculosis, and Non-Hodgkin's and Hodgkin's Disease*

An Evaluation of Differences

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The aim of this study was to identify characteristic features in bronchoalveolar lavage fluid (BALF) samples of patients with tuberculosis, non-Hodgkin's or Hodgkin's disease and to investigate whether these differences facilitate the distinction of those disorders from sarcoidosis presenting with a similar clinical picture. Nonsmoker patients with histologically verified sarcoidosis (n = 29), tuberculosis (n = 6) proven by positive culture, non-Hodgkin's disease, (n = 6) or Hodgkin's disease (n = 7), both histologically verified, were investigated by BAL. A control group consisted of subjects without any pulmonary history. The presence of CD4⁺ and CD8⁺ T lymphocytes, as well as the CD4/CD8 ratio in BALF, aided in the differentiation between

The use of bronchoalveolar lavage fluid (BALF) analysis for diagnostic purposes in pulmonary disorders has been widely established.¹⁻³ Previously, we reported the possibility of distinguishing between interstitial lung diseases, *ie*, sarcoidosis, extrinsic allergic alveolitis, and idiopathic pulmonary fibrosis by a number of selected variables derived from BALF analysis.⁴

In sarcoidosis, granuloma formation is preceded by a mononuclear cell alveolitis with increased numbers of activated T lymphocytes and alveolar macrophages.⁵⁻⁹ Although the lung is the most commonly affected organ, extrapulmonary manifestations, such as erythema nodosum, arthralgia, and hilar lymphadenopathy, constituting a clinical picture referred to as Löfgren's syndrome, frequently occur.^{10,11} Patients with Löfgren's syndrome, having the most severe alveolitis, show distinct characteristics in BALF sample analysis, among which are increased numbers of lymphocytes and high CD4/CD8 ratios.^{11,12}

Tuberculosis and malignant lymphomas, *ie*, non-Hodgkin's and Hodgkin's disease, especially the nodular-sclerosis type, also may present with bilateral mediastinal or hilar lymphadenopathy and alveolar mononuclear infiltration.^{13,14} These disorders, requiring an even more rapid

the various groups. Patients with malignant lymphomas had the lowest CD4/CD8 ratio in BALF, as well as in peripheral blood, and occasionally, plasma cells were present in BALF samples. The most important feature of BALF analysis in tuberculosis was detection of the causal microbial agent. In conclusion, although malignant lymphomas and tuberculosis require histologic evaluation and a positive culture, respectively, for diagnosis, BALF analysis may be of additional value in distinguishing those disorders from sarcoidosis.

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ANOVA = analysis of variance I

diagnosis and substantially different therapeutic regimens, should be readily differentiated from sarcoidosis.¹³⁻¹⁵

Recently, BALF sample analysis, in comparison with more conventional methods, has proven an even more sensitive technique in the diagnostic workup for tuberculosis detection.¹⁶⁻²⁰ In order to detect and further classify malignant lymphomas, histologic evaluation is required.^{14,21,22} However, obtaining representative tissue samples may be a major problem. Pulmonary localization of Hodgkin's disease has been confirmed by identification of Reed-Sternberg cells in the BALF specimen.²³⁻²⁶ Also, the detection of non-Hodgkin's disease by BALF evaluation, using immunologic markers, has been described.^{27,28}

The aim of this study was to investigate whether there are characteristic features in BALF samples obtained from patients with tuberculosis, non-Hodgkin's disease, or Hodgkin's disease and whether these differences assist in distinguishing these clinically similar disorders from sarcoidosis.

MATERIALS AND METHODS

Patients and Control Subjects

Bronchoalveolar lavage was performed in 90 sarcoidosis patients, 6 tuberculosis patients, 6 patients with non-Hodgkin's disease, and 7 patients with Hodgkin's disease. The control group consisted of 28 healthy individuals who did not have chest x-ray film abnormalities or history of pulmonary disease. All patients and control subjects were nonsmokers. The characteristics of the patients and control subjects are described in Table 1.

Our sarcoidosis patient population consisted of patients who had no

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Table 1—Characteristics of the Groups Studied:

Studied Groups	No. Cases	Age, yr	Sex	
			F	M
Control subjects	28	39 (19-70)	12	16
Sarcoidosis	29	43 (23-76)	14	15
Tuberculosis	6	41 (28-76)	2	4
Non-Hodgkin's disease	6	57 (39-71)	2	4
Hodgkin's disease	7	35 (16-79)	4	3

*Mean with range in parentheses.

symptoms (n = 11), those whose disease was detected on routine chest x-ray film, patients with respiratory and general constitutional symptoms (n = 50), and patients with Löfgren's syndrome (n = 29). All diagnoses were histologically proven. Unless otherwise stated, only the latter group was used in this comparative study.

The tuberculosis patient group consisted of six immunocompetent cases (five with pulmonary tuberculosis and one with lymph node tuberculosis). These patients initially presented with cough, dyspnea, erythema nodosum, chest pain, or fever. The chest x-ray film invariably showed infiltrates, pleural effusion, or enlarged mediastinal lymph nodes. Histologically, granulomas and necrosis were demonstrated. Five patients had proven infection with *Mycobacterium tuberculosis* and one patient, with *M bovis*.

All patients with malignant lymphomas initially presented with pulmonary manifestations. The chest x-ray films showed pulmonary infiltrates, interstitial involvement or enlarged mediastinal lymph nodes, or all three. All cases of non-Hodgkin's disease were histologically classified as low-grade B lymphocyte lymphomas of various stages (stages II, III, or IV) and the cases of Hodgkin's disease, as the nodular sclerotic type.¹⁴ The patients with Hodgkin's disease were in various stages of the disease (according to the Ann Arbor classification).^{14,15} At the time of performance of bronchoalveolar lavage (BAL), chemotherapy was not yet started.

Bronchoalveolar Lavage

The BAL was performed as previously reported during fiberoptic bronchoscopy.⁴ Simultaneously, blood samples were taken. In short, the procedure was as follows: After premedication with atropine and sometimes diazepam or codeine and locally anesthetizing the larynx and bronchial tree with 0.5 percent tetracaine, BAL was performed by standardized washing of the right middle lobe with four 50-ml aliquots of sterile saline solution (0.9 percent NaCl) at room temperature.

Sample Collection and Preparation

The first portion of lavage fluid recovered was collected in a special test tube which was sent for culture. After centrifugation, the sediment was screened for acid-fast bacilli by both fluorescent auramine-rhodamine B and Ziehl-Neelsen stains and cultures were performed on Löwenstein-Jensen medium.

Recovered BALF samples of the other three aliquots, kept on ice in a siliconized specimen trap, were centrifuged (5 min, 350 g) and separated from cellular compounds. Supernatants were directly stored at -70°C after an additional centrifugation step (10 min, 1,000 g). The cells were washed twice, counted, and suspended in minimal essential medium (Gibco, Grand Island, NY) supplemented with 1 percent bovine serum albumin (Organon, Teknika, Bostel, the Netherlands).

Preparations of the cell suspensions were made in a cytocentrifuge (Shandon). Cytospin slides of BALF sample cells were stained with May-Grünwald-Giemsa (Merck, Darmstadt, Germany) for cell differentiation. At least 1,000 cells were counted. Reed-Sternberg cells were recognized by scanty to moderate amounts of finely vacuolated cytoplasm and a multilobulated nucleus with vesicular chromatin and a large prominent eosinophilic-to-cyanophilic nucleolus.

If more than 15 percent lymphocytes were present, T lymphocyte

subpopulations were determined. Total numbers of T lymphocytes and subpopulations were recognized by staining with monoclonal antibodies CD3(OKT3), CD4(OKT4), and CD8(OKT8) from Ortho Pharmaceuticals (Diagnostic Systems, Beersse, Belgium). Identification of T lymphocytes reacting with monoclonal antibodies was performed by means of a conventional indirect immunofluorescence technique using FITC-labeled goat-antimouse-Ig (Nordic, Immunological Laboratories, Tilburg, the Netherlands and from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands). For the quantitative determination of albumin in serum samples and BALF samples, the albumin method was used. The albumin method is an adaptation of the bromocresol purple dye-binding method.^{29,30} In short this method is as follows. In the presence of a solubilizing agent, bromocresol purple, binds to albumin at pH 4.9. The amount of albumin-bromocresol purple complex is directly proportional to the albumin concentration. The complex absorbs at 600 nm. Albumin concentrations in serum and BALF samples were expressed in grams per liter and milligrams per liter, respectively.

Immunoglobulin concentrations, ie, IgM, IgG, and IgA in BALF samples were determined by an enzyme-linked immunosorbent assay method; microtiter plates were coated with a rabbit antihuman-isotype antiserum (anti-IgM [Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands] anti-IgG, or anti-IgA [Dako, Glostrup, Denmark]). Bound immunoglobulins from BALF samples were visualized by using a horseradish peroxidase-labelled rabbit-antihuman-immunoglobulin antiserum (with anti-IgA, anti-IgG, anti-IgM, anti-kappa, anti-lambda reactivity [Dako, Glostrup, Denmark]) and a chromogenic substrate orthophenyl diamine (Baker, Chemicals BV, Deventer, the Netherlands). Immunoglobulin concentrations in BALF samples were expressed in milligrams per liter using as a reference a commercial human standard serum, HOO-03 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands).

Statistical Analysis

Data are expressed as mean ± SEM and, if appropriate, as median with range. In order to detect statistically significant differences between the four patient groups, data were analyzed by the Kruskal-Wallis one-way analysis of variance (ANOVA) test. The Mann-Whitney U test was used for pairwise comparisons. Because 15 comparisons were made, a probability value smaller than 0.05/15 = 0.003 was considered statisti-

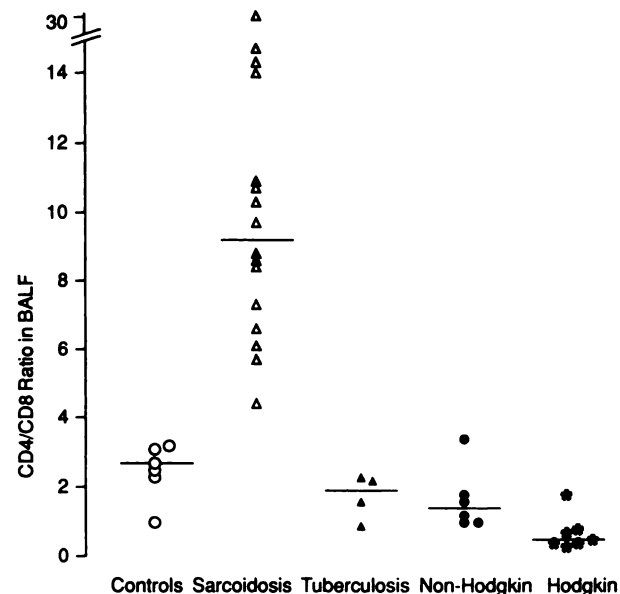


FIGURE 1. Individual CD4/CD8 T lymphocyte ratios (with median values) in BALF samples obtained from control subjects, patients with sarcoidosis, tuberculosis, non-Hodgkin's or Hodgkin's disease.

Table 2—Total Cell Count and Differential Cell Count in Bronchoalveolar Lavage Fluid Samples*

Groups	Total Cell Count, × 10 ⁶ /ml	Percentage of Total Cell Count†					
		AM	PMN	Lym	PC	Eos	MC
Control subjects	10.3 ± 1.5	89.8 ± 0.7	1.3 ± 0.2	8.4 ± 0.7	0.0 ± 0.0	0.44 ± 0.10	0.09 ± 0.03
Sarcoidosis	20.3 ± 1.7‡	60.3 ± 3.4‡	1.4 ± 0.2	37.9 ± 3.4‡	0.0 ± 0.0	0.32 ± 0.07	0.09 ± 0.03
Tuberculosis	26.9 ± 12.6‡	72.2 ± 7.6‡	1.2 ± 0.6	26.1 ± 7.8‡	0.0 ± 0.0	0.33 ± 0.21	0.15 ± 0.04
Non-Hodgkin's disease	23.9 ± 4.0‡	65.7 ± 5.2‡	2.5 ± 1.1	28.3 ± 3.1‡	2.5 ± 2.4‡	0.68 ± 0.49	0.33 ± 0.16‡
Hodgkin's disease	23.8 ± 7.4‡	65.2 ± 4.8‡	4.8 ± 2.2‡	28.1 ± 2.8‡	0.04 ± 0.04‡	1.54 ± 0.70	0.34 ± 0.16
p value§	0.85

*Data are expressed as mean ± SEM.

†AM = alveolar macrophages; PMN = polymorphonuclear neutrophils; Lym = lymphocytes; PC = plasma cells; Eos = eosinophils; MC = mast cells.

‡p < 0.04, Mann-Whitney versus control group.

§Kruskal-Wallis ANOVA test.

||p < 0.04, Mann-Whitney versus malignant lymphomas (non-Hodgkin's and Hodgkin's disease).

probability value smaller than 0.05/15 = 0.003 was considered statistically significant (Bonferroni's correction). Logistic regression analysis was used to discriminate between sarcoidosis and malignant lymphomas, given the CD4/CD8 ratios in BALF.

RESULTS

The cellular components and protein levels in BALF samples obtained from the patient groups and control subjects are summarized in Tables 2 to 5.

In the sarcoidosis group, the percentages of CD4⁺T lymphocytes were significantly higher, the percentage of CD8⁺T lymphocytes and mast cells lower, and the CD4/CD8 ratio in BALF samples higher, as compared with all other groups (Fig 1, Tables 2 to 4). The CD4/CD8 ratios in BALF samples of patients with Hodgkin's disease were decreased in comparison with all other groups including patients with non-Hodgkin's disease. Also, patients with either form of malignant lymphoma had significantly lower CD4/CD8 ratios in peripheral blood than sarcoidosis patients (Table 4). Subsequent comparisons between the four patient groups revealed most prominent differences in the percentages of CD4⁺T lymphocytes (p < 0.0001), CD8⁺T lymphocytes (p < 0.0001), and the CD4/CD8 ratio

(p < 0.0001) in BALF samples. The lowest BALF CD4/CD8 ratios were found in patients with Hodgkin's disease and the highest, in those with sarcoidosis. The CD4/CD8 ratios in the tuberculosis and non-Hodgkin's disease groups were similarly low (Fig 1, Table 4).

In two of the cases with low grade B lymphocyte lymphomas of low grade, one with paraproteins of the IgG-lambda and one of the IgM-kappa type, the diagnosis was made initially on BALF specimens. In the BALF samples of these latter patients plasma cells also were present. The IgM levels were lower in the sarcoidosis group, as compared with the non-Hodgkin's disease group, but the range was broad and the SEM was high, due to some cases with high IgM levels in BALF samples and due to the presence of paraproteins. Reed-Sternberg cells were identified in the BALF sample of one patient with Hodgkin's disease.

In tuberculosis patients, combined evaluation of Ziehl-Neelsen staining and culture for Mycobacterium species of BALF specimens yielded a sensitivity of 83.3 percent and a specificity of 100 percent, both of which were significantly higher than those obtained from sputum analysis (data not shown).

The ranges of the CD4/CD8 ratio in the BALF in pa-

Table 3—Absolute Number of Cells in Bronchoalveolar Lavage Fluid Samples of Control Subjects*

Groups	Cells†					
	AM	PMN	Lym	PC	Eos	MC
Control subjects	9.3 ± 1.4	0.13 ± 0.03	0.8 ± 0.1	0.0 ± 0.0	0.07 ± 0.01	0.01 ± 0.005
Sarcoidosis	12.1 ± 1.3§	0.27 ± 0.04§	7.9 ± 1.0§	0.0 ± 0.0¶	0.05 ± 0.02§	0.02 ± 0.01¶**
Tuberculosis	22.0 ± 11.7	0.31 ± 0.20	4.5 ± 1.2§	0.0 ± 0.0	0.04 ± 0.02	0.04 ± 0.02§
Non-Hodgkin's disease	14.8 ± 1.5§	0.61 ± 0.23§	7.2 ± 1.8§	1.0 ± 1.0§	0.14 ± 0.09	0.11 ± 0.07§
Hodgkin's disease	15.0 ± 0.5	1.06 ± 0.73§	7.3 ± 2.6§	0.01 ± 0.01§	0.32 ± 0.21	0.06 ± 0.04§
p value‡	0.35	0.16	0.20	0.07	0.58	0.02

*Data are expressed as mean absolute number of the total cell count × 10⁶/ml ± SEM.

†AM = alveolar macrophages; PMN = polymorphonuclear neutrophils; Lym = lymphocytes; PC = plasma cells; Eos = eosinophils; M = mast cells.

‡Kruskal-Wallis ANOVA test.

§p < 0.04, Mann-Whitney versus control group.

||p < 0.05, Mann-Whitney versus sarcoidosis.

¶p < 0.04, Mann-Whitney versus malignant lymphomas (non-Hodgkin's and Hodgkin's disease).

**p < 0.05, Mann-Whitney versus tuberculosis, non-Hodgkin's and Hodgkin's disease.

Table 4—Percentages of T Lymphocytes and T Lymphocyte Subpopulations in Bronchoalveolar Lavage Fluid Samples*

Groups	CD3	CD4	CD8	CD4/CD8 Ratio, BALF	CD4/CD8 Ratio, Peripheral Blood
Control subjects (n = 6)	73.0 ± 2.4 74 (68-81)	52.4 ± 3.4 49 (41-63)	19.0 ± 1.4 20 (14-34)	2.6 ± 0.2 2.7 (1.0-3.0)	1.8 ± 0.3 1.8 (1.1-2.9)
Sarcoidosis (n = 16)	88.7 ± 2.0‡ 91 (63-95)	80.3 ± 2.7‡ 85 (48-90)	8.9 ± 0.8‡ 8 (3-15)	10.7 ± 1.5‡ 9.3 (4.4-30)	2.6 ± 0.4‡‡ 2.2 (1.1-6.5)
Tuberculosis (n = 4)	67.7 ± 10.5§ 71 (48-84)	42.0 ± 7.6¶ 47 (21-53)	27.8 ± 7.6¶†† 28 (9-45)	1.8 ± 0.3‡** 1.9 (0.9-2.3)††	1.9 ± 0.3 1.6 (1.5-2.5)
Non-Hodgkin's disease (n = 6)	83.0 ± 6.9 91 (54-99)	50.0 ± 4.6** 53 (34-61)	34.2 ± 4.9‡** 34 (18-54)	1.7 ± 0.4‡** 1.4 (1.0-3.4)	1.2 ± 0.3¶ 1.1 (0.8-2.2)
Hodgkin's disease (n = 7)	81.6 ± 3.2 79 (70-96)	30.7 ± 4.2‡§** 32 (21-53)	52.0 ± 4.9‡§** 55 (29-67)	0.7 ± 0.2‡§** 0.5 (0.3-1.8)	1.1 ± 0.5¶ 1.0 (0.2-2.2)
p value†	...	<0.0001	<0.0001	<0.0001	...
Pooled sarcoidosis population (n = 77)	87.8 ± 0.9† 90 (63-99)	70.6 ± 1.8‡ 80 (33-97)	16.3 ± 1.4‡ 12 (2-61)	8.1 ± 0.9‡ 6.3 (0.5-48.5)	2.3 ± 0.4‡‡ 2.1 (0.4-6.5)

*Data are expressed as mean ± SEM; and median with range in parentheses.

†Kruskal-Wallis ANOVA test.

‡p < 0.04, Mann-Whitney versus control group.

§p < 0.05, Mann-Whitney versus non-Hodgkin's disease.

||p < 0.001, Mann-Whitney versus malignant lymphomas (non-Hodgkin's and Hodgkin's disease).

¶p < 0.04, Mann-Whitney versus sarcoidosis.

**p < 0.001, Mann-Whitney versus sarcoidosis.

††p < 0.04, Mann-Whitney versus Hodgkin's disease.

‡‡p < 0.01, Mann-Whitney versus malignant lymphomas (non-Hodgkin's and Hodgkin's disease).

tients with Löfgren's syndrome (4.4 to 30.0) and in patients with non-Hodgkin's or Hodgkin's disease (0.3 to 3.4) are disjoint (Fig 1, Table 4). Thus, the CD4/CD8 ratio may serve as a perfect testing variable with 100 percent sensitivity and specificity for distinguishing malignant lymphomas with pulmonary involvement from Löfgren's syndrome. In order to test this observation, a logistic regression analysis was performed including all 77 sarcoidosis patients (disregarding the clinical presentation), wherein T lymphocyte subpopulations were determined, and all patients with either malignancy (n = 13). The CD4/CD8 ratios were divided into three intervals, *ie*, values between 0 and 0.54 (interval A), between 0.54 and 3.39 (B), and those between 3.39 and 48.5 (C). Interval A contained 4 patients, all with malignant lymphomas, and interval B included 9 patients with lymphomas and 23 with sarcoidosis. In interval C, only sarcoidosis patients (n = 54) were found. Each unit increase of the CD4/CD8 ratio decreases the odds of lymphomas by a factor of 0.30 (p < 0.00005). Based on the present data, the diagnosis of malignant lymphoma was considered probable when a CD4/CD8 ratio below 1.85 was found. The sensitivity equals 12 of 13 or 92.3 percent and the specificity, 64 of 77 or 83.1 percent.

DISCUSSION

Although bilateral mediastinal or hilar lymphadenopathy is most frequently caused by the benign and self-limiting disease sarcoidosis, disorders that require rapid diagnosis such as tuberculosis and malignant lymphoma should be excluded.^{13,14} In the present study, differences in BALF cell profile and protein levels between patients suffering from sarcoidosis, tuberculosis, non-Hodgkin's or Hodgkin's disease were found.

As did Harf et al,¹⁹ we observed high proportions of mast cells in BALF samples in tuberculosis, in contrast to sarcoidosis. In addition, CD4/CD8 ratios were lower in comparison with those of sarcoidosis patients and control subjects, which was in agreement with the findings of others.³¹ The cell-mediated immune response to M tuberculosis, which plays a predominant role in host defense, involves subpopulations of specifically sensitized CD4¹ helper-inducer or cytolytic T lymphocytes.^{31,32} An initially increased number of lymphocytes is a feature of the histopathology of pulmonary tuberculosis with a CD8¹ T

Table 5—Protein Levels in Bronchoalveolar Lavage Fluid Samples of Control Subjects and Patients*

Groups	Lavage Albumin	IgM	IgM/Lavage Albumin	IgG	IgG/Lavage Albumin	IgA	IgA/Lavage Albumin
Control subjects	71 ± 8.5	0.4 ± 0.1	0.01 ± 0.002	11.1 ± 2.0	0.16 ± 0.02	3.6 ± 0.7	0.05 ± 0.01
Sarcoidosis	140 ± 20.9†	1.9 ± 1.3‡	0.01 ± 0.0‡	46.5 ± 12.3†	0.35 ± 0.04†	10.4 ± 3.2†	0.07 ± 0.01
Tuberculosis	106 ± 29.9	1.3 ± 0.7	0.01 ± 0.01	35.0 ± 8.9†	0.37 ± 0.07†	10.7 ± 2.8†	0.12 ± 0.05†
Non-Hodgkin's disease	163 ± 54.3†	8.8 ± 6.9	0.08 ± 0.07	287 ± 173†	1.47 ± 0.90†	23.2 ± 11.2†	0.19 ± 0.10
Hodgkin's disease	182 ± 45.0†	3.1 ± 2.0	0.01 ± 0.01	99.2 ± 38.7†	0.48 ± 0.11†	19.3 ± 8.9	0.09 ± 0.04

*Data are expressed as mean (milligrams per liter) ± SEM.

†p < 0.01, Mann-Whitney versus control group.

‡p < 0.05, Mann-Whitney versus malignant lymphomas (non-Hodgkin's and Hodgkin's disease).

lymphocyte predominance, whereas during recovery, a CD4⁺ predominance is found.³¹ The CD8⁺ T lymphocytes are believed to be involved in the production of 1,25(OH)₂D₃.³³ This compound has been implicated in the improvement of the mycobacterial killing capacity of alveolar macrophages. In tuberculosis, CD4⁺ T lymphocytes rather than CD8⁺ T lymphocytes express receptors for 1,25(OH)₂D₃, whereas a greater proportion of CD8⁺ than of CD4⁺ T lymphocytes in patients with sarcoidosis are 1,25(OH)₂D₃ receptor-positive.³³⁻³⁵ Thus, the various distribution of 1,25(OH)₂D₃-receptors points to a different role for the potent immunoregulatory molecule in the granulomatous inflammatory reactions in sarcoidosis and tuberculosis, respectively.³³ The diagnosis of tuberculosis can only be confirmed by culture. In this study, combined evaluation of Ziehl-Neelsen staining and culture for Mycobacterium species of BALF specimens was more sensitive and specific than that of sputum, which was in agreement with studies by others.¹⁶⁻¹⁸

Lymphocytic lymphomas are immunologically defined by the monoclonal proliferation of T or B lymphocytes.¹⁴ Tumor cells derived from B lymphocytes produce immunoglobulins of one single light chain type.³⁶ The majority of lymphomas with pulmonary manifestations are non-Hodgkin's disease derived from B lymphocytes.^{14,21,22} However, to date, the diagnostic value of BALF cellular analysis in malignant lymphomas has not been established. In our study, the cellular BALF profile differed between sarcoidosis patients and patients with non-Hodgkin's or Hodgkin's disease. All patients with either malignant lymphoma showed a lymphocytosis in BALF samples. However, a high proportion of lymphocytes is not a characteristic finding, since this has been found in BALF specimens in many pulmonary disorders.³ The presence of plasma cells in BALF was found to be highly suggestive for malignant lymphomas, especially for non-Hodgkin's disease with paraproteins in their BALF samples. Recently, plasma cells in BALF were associated with extrinsic allergic alveolitis and other antibody-mediated inflammatory processes of the lung, as well as with non-Hodgkin's disease.³⁷ Increased proliferation of B lymphocytes has been found in lymphocytic lymphomas,^{14,36,38} which may account for the presence of plasma cells in BALF samples of patients with malignant lymphomas. Also, in these patients, paraproteins were detected (data not shown). Therefore, our results indicate that BALF studies (B lymphocyte marker and paraprotein analysis) to detect monoclonality can be of additional value in distinguishing between malignant lymphomas and other pulmonary disorders in patients with plasma cells present in BALF samples.

The most important characteristic features in BALF, which allowed the differentiation between malignant lymphomas and sarcoidosis, were differences in T lymphocyte subpopulations and the CD4/CD8 ratios. Moreover, patients with malignant lymphomas, in particular patients with Hodgkin's disease, also demonstrated a decreased

CD4/CD8 ratio in peripheral blood, most likely as a consequence of an advanced, disseminated disease.¹⁴ A permanent immunologic defect, both in number and function of T lymphocytes, has been reported to be a concomitant of Hodgkin's disease.¹⁴ However, occasionally, also low CD4/CD8 ratios in BALF were found in sarcoidosis patients.

In this study, the number of mast cells were high in the BALF in patients with tuberculosis and those with malignant lymphomas, in contrast to patients with active sarcoidosis. Recently, Pesci et al³⁹ suggested that mast cells participate in chronic inflammation and that their presence is related to interstitial fibrosis in fibrotic lung disorders. Therefore, in addition to assessing CD4/CD8 ratios, determinations of other BALF constituents, such as plasma cells, mast cells, and immunoglobulins, may provide additional information to discriminate among the studied disorders besides the CD4/CD8 ratios.⁴⁰⁻⁴³

Although the patient populations in this study are small, the study illustrates that a limited invasive technique, such as BAL, may be of additional value to distinguish between sarcoidosis and other disorders with similar clinical manifestations, such as tuberculosis and malignant lymphomas with pulmonary involvement, provided that simultaneous careful clinical and pathologic staging is performed. The CD4/CD8 ratio may facilitate the differentiation between sarcoidosis, tuberculosis, and malignant lymphomas. In addition, the presence of plasma cells in BAL fluid may permit detection of malignant lymphomas, highly likely to be non-Hodgkin's disease. Future BALF studies, including immunologic marker analyses, are needed to investigate the reliability of BAL in diagnosing malignant lymphomas with pulmonary involvement.

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