

Predictive value of bronchoalveolar lavage cell analysis in sarcoidosis

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ABSTRACT Patients with histopathologically proved sarcoidosis were studied serially by means of bronchoalveolar lavage, initially at the time of diagnosis and then six and 12 months later. Two years later they were evaluated by chest radiography and lung function tests and classified in terms of recovery or progression over the previous two years. The recovery of lymphocytes and granulocytes in lavage fluid was of limited prognostic value for persistent lung disease. In contrast, patients with increased numbers of mast cells recovered by lavage were more likely to deteriorate. Significantly increased mast cell counts ($\geq 0.5\%$ of total cells recovered) were seen in at least one lavage investigation in 15 of the 16 patients with more active and progressive disease, but in only eight of 23 patients with inactive disease ($p < 0.001$). A persistent increase of mast cells on serial measurement occurred in nine of the 16 patients with active disease and in four of the 23 patients in whom the disease was inactive ($p < 0.02$). The finding in the two subsequent lavages of lymphocytosis (lymphocytes $> 30\%$ of recovered cells) or neutrophilia (neutrophils $> 15\%$) combined with mastocytosis (mast cells $\geq 0.5\%$) occurred in nine of the 16 patients with active disease but in no patients with inactive disease.

The technique of bronchoalveolar lavage has been used extensively since it was first introduced in the early 1970s¹ and the findings have contributed to the understanding of basic cellular mechanisms in various interstitial lung diseases.^{2,3} Several studies have shown good agreement between the findings in lavage fluid investigations and histopathology of lung biopsy specimens.^{4,5} In sarcoidosis the prominent finding in the lavage fluid is substantial lymphocytosis, mainly reflecting an increase of T lymphocytes of the OK T4/Leu3 T helper cell phenotype.⁶ An increase of lavage T lymphocytes with a positive pulmonary gallium-67 scintigram has been claimed to be a sign of high intensity alveolitis, possibly predicting progressive lung disease.^{7,8} Other investigators have disputed the prognostic value of lavage fluid lymphocytosis, especially when conclusions are drawn from a single lavage sample.^{9,10} A persistent increase of lavage lymphocytes in serial measurements has been reported to be a more reliable estimate of disease activity.¹¹ Increased numbers of polymorphonuclear neutrophils¹² and mast cells^{13,14} in lavage fluid from patients

with sarcoidosis have been reported, by us and others, as possible indices of disease activity. Recently, however, the clinical value of lavage was questioned and it was concluded that the value of cell counting in lavage fluid still remains to be proved.¹⁰

The present study was designed in an attempt to assess the prognostic value of lymphocyte, macrophage, neutrophil, and mast cell counts in lavage fluid from patients with sarcoidosis. Another aim was to determine whether additional prognostic information may be obtained by repeated lavages. In parallel to these lavage studies we investigated changes in lung function and radiographic appearances. The results suggest that the presence of mastocytosis in sequential lavage fluid samples is a prognostically unfavourable finding.

Methods

We studied 45 patients (26 women, 19 men) with histopathologically proved sarcoidosis. Their mean age was 47 (range 28-74) years, and five were smokers. All were newly diagnosed and were admitted to our clinic for initial investigation.

The potential study population comprised a total of 53 patients. Five were excluded at the initial investi-

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gation because of extrathoracic symptoms (hypercalcaemia (2), myocardial sarcoidosis (1), aggressive uveitis (1), neurosarcoidosis (1)) requiring systemic corticosteroid treatment. One patient was excluded because of asthma, another because of mental disturbances that would have made evaluation of the clinical course difficult. One patient developed acute myeloid leukaemia. As controls we used 21 healthy, non-smoking volunteers with a mean age of 32 (range 21–57) years.

Patients were investigated initially and at follow up studies after six and 12 months by the performance of bronchoscopy with bronchoalveolar lavage, pulmonary function tests, and chest radiography. After two years the patients were evaluated according to symptoms, radiographic findings, and pulmonary function. The chest radiographs were reviewed by an experienced physician without knowledge of the data on lavage fluid or other clinical aspects of the patients. On the basis of radiographic and functional criteria at the two year follow up investigation patients were divided into the following subgroups: (1) Complete recovery (CR), defined as patients ($n = 14$) with no symptoms and with a normal chest radiograph and normal lung function. (2) Inactive disease with sequelae (IAS), defined as patients ($n = 9$) with radiographic improvement during the first year and with stationary radiographic findings and lung function during the second year. They had persistent abnormalities, either radiographic infiltrates or a persistently low carbon monoxide transfer factor (TLCO) or vital capacity (VC)—that is, $< 75\%$ or $< 80\%$ respectively of predicted normal values ($> 2SD$ below the values from a normal reference population), or a reduction in TLCO or VC of over 15% below the initial values. (3) Persistently active disease (PA), defined as patients ($n = 16$) who showed signs of disease activity during the last year of the 2 year follow up period, either with appearance or increase of parenchymal infiltrates ($n = 12$) or a reduction of VC or TLCO $> 15\%$ ($n = 11$) compared with the findings at 12 months. (4) Rapidly progressive disease (RP): six of the patients developed symptoms and signs of rapidly progressive lung dis-

ease soon after initial assessment, with increasing parenchymal infiltrates and substantial deterioration of lung function. According to local therapeutic practice corticosteroid treatment was initiated within six months of the initial evaluation in these subjects. Only the pretreatment lavage data on these patients were used for statistical analysis.

Vital capacity was measured by standard spirometry and transfer factor for carbon monoxide by the single breath technique. The values obtained were expressed as percentages of predicted normal values.^{15,16} The following radiographic criteria were used for staging: stage 0—no abnormality; stage I—bilateral hilar lymphadenopathy alone; stage II—bilateral hilar lymphadenopathy with parenchymal infiltrates; stage III—parenchymal infiltrates without hilar lymphadenopathy. The lung function and radiographic findings in our patients after a two year follow up are summarised in table 1.

Before bronchoscopy patients and controls were given subcutaneous atropine or scopolamine, usually combined with morphine or pethidine chloride. The upper respiratory tract was anaesthetised with lignocaine hydrochloride. A fiberoptic bronchoscope (Olympus BF IT or BF 4B2, Tokyo) was wedged in the segmental bronchus of the lingula and 240 ml sterile Krebs-Ringer phosphate buffer, pH 7.3 at 37°C, was infused in boluses of 60 ml. The fluid was gently aspirated immediately after each instillation. The lavage fluid was kept on ice and filtered through a nylon filter (pore diameter 100 μm , Syntab Product AB, Malmö, Sweden). The cells were then collected by centrifugation at 400 g for 15 minutes. The cells were gently resuspended in balanced salt solution to a concentration of 10^6 cells/ml, with epithelial cells excluded. Preparations for cytological studies were made in a cytocentrifuge (Cytospin Shandon, Southern Products Ltd, Runcorn, UK) with 5×10^4 non-epithelial cells a slide. The cytocentrifuge preparations were stained with May-Grünwald-Giemsa before differential counting. Mast cells were stained with acid toluidine blue and counterstained with Mayers acid haematoxylin.¹⁷ Lymphocytes, polymorphonuclear

Table 1 Radiological pulmonary findings and mean (SD) lung function values in patients with sarcoidosis at a two year follow up

Patient group	Radiographic stage (n)				Vital capacity (% pred)	Transfer factor (% pred)
	0	I	II	III		
Complete recovery (n = 14)	14	0	0	0	99 (12)	93 (13)
Inactive disease with sequelae (n = 19)	6	0	0	3	98 (7)	80 (14)
Persistently active disease (n = 16)	1	1	1	13	93 (8)	70 (11)

cells, and macrophages were expressed as percentages of 200 non-epithelial cells counted. The relative counts of these cells were normally distributed. The numbers of mast cells in 10 visual fields with $\times 16$ magnification were expressed as percentages of all non-epithelial cells. The mast cell counts were log normally distributed.

The study was performed according to the Declaration of Helsinki and with free and informed consent of all volunteers and patients. For statistical comparison between the groups we used Wilcoxon's rank sum test. Chi-squared analysis was used for cross tabulation tests. Fisher's exact test was used when the expected frequency was less than 5.

Results

LAVAGE CELL ANALYSIS

The lavage findings for the four subgroups of patients and for the healthy controls are presented in table 2. Lavage lymphocytosis was present in all subgroups of patients at the initial investigation. The degree of lymphocytosis was similar in the CR, IAS, and PA groups, but patients with rapidly progressive disease had significantly higher lymphocyte counts than the other groups of patients ($p < 0.05$). In the CR group the lymphocytosis was less at the follow up investigations. The recovery of alveolar macrophages at the initial investigation was similar in all the subgroups of patients. In the CR group the macrophage counts were more normal at the follow up assessments as the lymphocyte counts declined. The recovery of neutrophils varied considerably between patients but no difference between subgroups were seen.

The number of mast cells recovered by lavage was the variable that best discriminated between the subgroups of patients (table 2). The percentages of mast cells recovered were in the order RP > IAS > CR > healthy controls ($p < 0.001$). The lavage fluid mastocytosis had decreased in the CR and IAS groups at the 6 and 12 month follow up investigations but was unchanged in the PA group.

EVALUATION OF THE PROGNOSTIC VALUE OF ABNORMAL LAVAGE CELL FINDINGS

In an attempt to analyse the prognostic clinical value of abnormal lavage cell findings we defined significant lymphocytosis of the lavage fluid as $>30\%$ lymphocytes, neutrophilia as $>15\%$ neutrophils, and mastocytosis as $\geq 0.5\%$ of total cell counts. We also analysed the sensitivity and specificity of single and serial lavage cell counts in the prediction of deterioration to elucidate the validity of abnormal lavage cell findings in determining prognosis.

At the initial investigation most patients in all subgroups had lavage fluid lymphocytosis (table 3). Lavage fluid mastocytosis was seen in most patients from the PA group and in about one third of patients from the CR and IAS groups. The six patients who developed rapidly progressive disease were only investigated once, before the start of steroid treatment; all six had lymphocytosis of the lavage fluid, five mastocytosis, and one neutrophilia (table 3).

We analysed the data to determine the prognostic value of abnormal lavage cell findings on at least one occasion during the first year after diagnosis (table 3). Lavage fluid lymphocytosis was found at least once during this period in more than 60% of all patients (patients in the RP group excluded) and it had no prognostic value. Neither did lavage fluid neutrophilia predict the outcome. In contrast, lavage mastocytosis was associated with deterioration in lung disease. However, lavage mastocytosis occurred at least once during the first year after diagnosis in 4/14 patients with complete recovery suggesting a low degree of specificity for deterioration of lung function.

Finally, we assessed the prognostic value of abnormal cell findings present at two subsequent investigations (table 3). Lavage fluid mastocytosis in serial lavages was found in only one of the 14 patients in the CR group and in nine of the 16 patients in the PA group. Certain prognostic information was also provided by lavage fluid lymphocytosis. The combination of the lymphocytosis or neutrophilia of the lavage

Table 2 Bronchoalveolar cell counts in lavage fluid from patients with sarcoidosis: median values and ranges obtained at the first investigation and six months and 12 months later

Patient group (n)	Time (months)	Total cells ($\times 10^3/l$)	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Mast cells (%)					
Complete recovery (14)	0	6.7	3.0-23.0	52	14-91	44	8-85	1	0-5	0.16	0.00-5.37
	6	5.4	3.0-41.6	71	22-88	24	10-61	2	0-18	0.11	0.03-1.09
	12	9.0	2.4-32.6	84	15-95	11	5-83	3	0-18	0.07	0.00-0.46
Inactive disease with sequelae (9)	0	7.5	2.0-13.0	67	24-90	31	7-55	1	0-2	0.40	0.03-4.63
	6	8.4	2.6-12.3	70	41-90	36	9-56	1	0-4	0.34	0.03-1.89
	12	6.0	3.4-10.8	68	40-93	30	7-59	2	0-4	0.23	0.00-2.23
Persistently active disease (16)	0	9.0	2.0-27.0	51	19-87	46	10-77	1	0-24	0.63	0.03-9.43
	6	7.0	2.0-18.3	39	10-88	56	12-87	1	0-54	0.63	0.06-3.77
	12	5.0	1.6-14.8	53	24-76	44	5-72	2	0-35	0.62	0.03-4.00
Rapidly progressive disease (6)	0	13.0	5.0-20.0	33	21-42	63	45-78	1	0-18	0.93	0.06-8.29
Healthy controls (21)		6.4	2.4-10.0	92	68-98	6	2-30	1	0-5	0.00	0.00-0.02

Table 3 Frequency of abnormal lavage cell findings in patients with sarcoidosis (classified according to outcome after two years): (A) at initial investigation, (B) once or more during the first year after diagnosis, and (C) at two subsequent investigations during the first year

	Complete recovery (n = 14)	Incomplete recovery (n = 9)	Persistently active disease (n = 16)	Rapidly progressive disease (n = 6)	p*
A Abnormal lavage cell findings at the initial investigation (No (%) of patients)					
Lymphocytes > 30%	10 (71)	7 (78)	12 (75)	6 (100)	NS
Neutrophils > 15%	0	0	1 (6)	1 (17)	NS
Mast cells \geq 0.5%	3 (21)	3 (33)	9 (56)	5 (83)	NS
B Abnormal lavage cell findings once or more during the first year after diagnosis (No (%) of patients)					
Lymphocytes > 30%	10 (71)	6 (66)	14 (88)		NS
Neutrophils > 15%	2 (14)	0	4 (25)		NS
Mast cells \geq 0.5%	4 (29)	4 (49)	15 (94)		< 0.001
C Abnormal lavage cell findings at two subsequent investigations during the first year after diagnosis (No (%) of patients)					
Lymphocytes > 30%	3 (21)	3 (33)	9 (64)		< 0.05
Neutrophils > 15%	1 (17)	0	1 (6)		NS
Mast cells \geq 0.5%	1 (7)	3 (33)	9 (64)		< 0.02

*Comparison of complete recovery with incomplete recovery and persistently active disease groups (Fisher's exact test).

fluid with pulmonary mastocytosis at two subsequent measurements had the highest specificity (100%) for the prediction of deterioration.

Discussion

The recovery of lymphocytes and granulocytes during bronchoalveolar lavage in our hands has proved to be of limited prognostic value for lung disease in sarcoidosis. We observed, however, that lavage fluid lymphocytosis that was still present at two subsequent investigations tended to correlate with deterioration of lung function, which might support the conclusions of those who have suggested that lavage fluid lymphocytosis, especially when found in serial measurements, is of clinical value^{7,8,11}. Possibly T cell subpopulation studies might have provided different information. Although an increased recovery of neutrophils in lavage fluid has been proposed as reflecting more active sarcoidosis,¹⁸ we were unable to relate neutrophilia to aggressive disease. This study did, however, support our preliminary observation that an increase of mast cells in the lavage fluid seems to be related to more active sarcoidosis of the lungs.¹³

In previous studies of lavage fluid from patients with sarcoidosis mast cells have accounted for 0.2–1.4% of the total cells recovered.⁹ Haslam *et al*¹⁹ found a lower proportion of mast cells, less than 0.5% in all 40 patients. In our study the significant increase of mast cells in lavage fluid—that is, \geq 0.5% of the total cells recovered—was a frequent finding that was observed in about half the patients in at least one lavage fluid out of three during the first year of the disease. Thus finding mastocytosis in a single lavage seems to be of limited value as a prognostic sign. The specificity of lavage fluid mastocytosis as an indicator of persistently active lung disease increased, however, when two

subsequent lavages with increased mast cells were recorded. The combination in two subsequent lavages of mastocytosis and lymphocytosis (> 30% of total cells recovered) or mastocytosis and neutrophilia (> 15% of total cells recovered) was seen in nine of the 16 patients with persistently active disease, but in none of the 14 patients with complete recovery or the nine with inactive disease with sequelae. The possible link between lavage fluid mastocytosis and sarcoidosis of the lung was further supported by our observation that the largest number of mast cells at the initial investigation was found in those patients who had rapidly progressive lung disease requiring corticosteroid treatment shortly after the diagnosis of sarcoidosis.

The present observation that an increase in mast cell numbers is related to the likelihood of progression of lung disease merits further study of the possible pathophysiological role of the mast cell. Previous investigators have reported that mast cells appear in increased numbers in the lung tissue of patients with fibrotic lung diseases.²⁰ It has also been observed in animal experiments that the number of lung mast cells increases with increasing severity of pulmonary fibrosis.^{21,22} We ourselves have recently reported that bronchoalveolar mastocytosis in sarcoidosis and other interstitial granulomatous lung diseases is closely linked to biochemical signs of fibroblast activation.^{23,24} Furthermore, histamine stimulates fibroblast proliferation in vitro and mast cell degranulation in vivo is associated with H₂ receptor dependent proliferation of fibroblast like cells.^{25,26} Thus previous observations and the present study suggest that mast cells and mast cell activation may be related to lung tissue damage. The link between recruitment of mast cells to the lung and the established cellular immune events in the lung in sarcoidosis is not clear. It is,

however, apparent that T lymphocytes have a role in mast cell proliferation²⁶ and modulate mast cell function.^{26,27}

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