(BOOP), human immune deficiency virus (HIV) infected patients and drug induced pneumonitis.

It is worth mentioning that the presence of very high percentages of lymphocytes in association with increases in mast cells >1% might represent a diagnostic indicator of EAA [22]. Of course, this combination is only of value in cases which are currently, or have been recently, exposed to antigen since mast cells return to the normal range within one to three months after removal from exposure.

The pattern of alveolitis in EAA during the follow-up

Although it is difficult to precisely separate patients on the basis of antigen exposure and, thus, correctly subdivide EAA cases into strictly defined groups, a distinction needs to be made between patients who continue to be exposed to antigens and patients who had been removed from the antigenic exposure.

Concerning those patients who continue to be exposed to antigens, several authors have shown a decrease (percentage or absolute) of lymphocytes during the follow-up [137, 138] while other authors have demonstrated that the increase of the total number of lymphocytes was a persistent feature in EAA patients still exposed to relevant antigens [139]. With regard to immunological surface markers, a recovery of the CD4/CD8 ratio has been evidenced during the follow-up only in those patients who had been removed from further antigen exposure [138, 140], thus suggesting that the immunological abnormalities in these patients progress towards normal. Note that the behaviour of the CD4/CD8 ratio is not consistent in all cases. A recovery of the CD4/CD8 ratio was not found in subjects still exposed to relevant antigens [141].

As far as clinical management is concerned, studies performed on this topic have indicated that there is no correlation between radiographic changes, pulmonary function, BAL findings or levels of precipitating antibodies and different phases of the disease [141–144].

Asymptomatic EAA patients

Although in asymptomatic EAA patients the increase of lymphocytes (mostly CD8+ cells) with respect to controls is less prominent, the data are qualitatively similar to those observed in symptomatic patients [112, 127, 143]. Data indicating that an alveolitis similar to that observed in EAA patients develops in asymptomatic patients raises the question of how, when and why clinical features become apparent. The answer, however, still remains inconclusive.

Analysis of humoral constituents of BAL

The analysis of humoral constituents of BAL does not significantly improve the diagnosis of patients with EAA, as compared to the great value of the BAL cytology and immunocytology in the clinical assessment of this disease. However, the evaluation of hyaluronate and type III procollagen peptide concentrations in BAL might be useful in monitoring the disease [60, 145].

Table 1. – Evolution of alveolitis in patients with extrinsic allergic alveolitis

Time from acute episode	Type of reaction	BAL findings
4–72 h	mediated by immune complexes	increase of neutrophils mast cells plasma cells
3rd day to weeks	mediated by suppressor/ cytotoxic lymphocytes	increase of CD8+ cells
Several months	delayed type hypersensitivity	increase of CD8+ cells CD4+ cells

Occupational lung diseases due to inhalation of inorganic dust

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This chapter aims to review the clinical use of BAL in patients with interstitial lung disease (ILD) associated with occupational or environmental exposure to inorganic dust and minerals. Excluded from this paper are occupational asthma and ILD due to inhalation of organic dusts (extrinsic allergic alveolitis).

Indications for performing a BAL in ILD associated with inorganic dust exposure are: 1) the exclusion of other causes of ILD, such as sarcoidosis, pulmonary haemorrhage syndromes, malignancies *etc.*, in patients additionally exposed to inorganic dust; 2) the documentation of mineral dust exposure in patients who may not be aware of being at increased risk of dust inhalation; 3) the documentation of the local immune and inflammatory reaction, *i.e.* the alveolitis.

Table 1. – How many subjects show signs of alveolitis

Authors	[Ref.]		Type	Increased lymphocytes		Increased neutrophils	
Asbestosis (ASB) and asbestos exposure (EXP)							
Gellert	[35]	1985	ASB	8/27	(29%)	13/27	(46%)
Xaubet	[158]	1986	EXP	0/15	(0%)	0/15	(0%)
			ASB	0/27	(0%)	22/27	(81%)
Robinson	[154]	1986	ASB	3/27	(11%)	20/27	(74%)
Rom	[151]	1987	ASB	2/18	(11%)	6/18	(33%)
Haslam	[159]	1987	ASB	0/19	(0%)	12/19	(63%)
Costabel	[146]	1990	EXP	10/29	(34%)	9/29	(31%)
			ASB	7/35	(20%)	9/35	(26%)
Silicotic disease (SIL) and exposure (EXP)							
Christman	[49]	1985	EXP	5/9	(56%)	0/0	(0%)
Rom	[151]	1987	SIL	1/6	(17%)	1/6	(17%)
			CWP	0/15	(0%)	6/15	(40%)
Costabel	[146]	1990	MDP	9/26	(35%)	11/26	(42%)

The table shows the numbers and percentages of patients with an increase in the given cell type above the normal range of the individual authors' laboratories. CWP: coal workers' pneumoconiosis; MDP: mixed dust pneumoconiosis.

Table 2. – Mean values of BAL cell differentials

Author	[Ref.]		Type	Lympho %	Neutro %	Eo %	CD4/CD8
Asbestosis (ASB) and asbestos exposure (EXP)							
Gellert	[35, 157]	1985	ASB	11%	17%	5%	1.0
Xaubet*	[158]	1986	EXP	normal	normal	normal	
			ASB	normal	8±5	normal	
Robinson	[154]	1986	ASB	normal	7±1	2±0.4	
Spurzem	[156]	1987	EXP+ASB	30±5	2±1	normal	
Rom	[151]	1987	ASB	21±4	3±1	normal	
Haslam	[159]	1987	ASB	normal	5%	4%	
Wallace	[148]	1989	EXP	19±3	normal	normal	2.9±0.6
Costabel	[146]	1990	EXP	17±4	8±4	1±1	2.4±0.4
			ASB	8±2	5±2	1±1	
Silicotic disease (DIS) and exposure (EXP)							
Christman	[49]	1985	SIL-EXP	16±4	normal	normal	
Begin	[150]	1987	SIL-EXP	normal	normal	normal	
			SIL-DIS	16±4	normal	normal	
Robalo-Cordeiro*	[147]	1988	SIL-DIS	14±10	7±5	normal	0.8±0.1
Rom	[151]	1987	SIL-DIS	22%	4%	normal	
			CWP-PMF	normal	4%	normal	
Wallaert	[152]	1990	CWP-PMF	normal	3±1	normal	
Araujo*	[149]	1986	MDP-DIS	26±12	normal	normal	0.9±0.4
Costabel	[146]	1990	MDP-DIS	12±3	4±2	normal	1.1±0.2

*: data are mean±SD; SIL: silicosis; CWP: coal workers' pneumoconiosis; MDP: mixed dust pneumoconiosis; PMF: progressive massive fibrosis; Lympho: lymphocytes; Neutro: neutrophils; Eo: eosinophils.

BAL findings

Inflammatory cell profiles

The total number of cells recovered is either normal [49, 146–148] or slightly increased [149–152]. As in other types of ILD it is important to correct the total cell count for smoking habits [153].

Usually, the severity of the alveolitis in patients with occupational dust exposure is mild in intensity. Many patients show a normal BAL cell profile (table 1). In those patients who have a relative increase in lymphocytes and/or neutrophils, the increase is moderate when looking at the mean of values of different study groups so far reported in the literature (table 2), except for those with chronic beryllium disease.

The type of alveolitis, whether associated with a lymphocytic or a neutrophilic/eosinophilic predominance, or with an increase in both, is summarized in table 2. In patients with lone increase in neutrophils caution must be taken regarding the diagnostic interpretation, since moderate increases can arise in chronic bronchitis, in particular in smokers, which has a high incidence in this population.

Asbestos disorders. In subjects with known exposure to asbestos, but without radiographic or functional signs of ILD, the most frequent finding is a lymphocytic alveolitis. In fact, in this group of subjects, the mean values of BAL lymphocytes, range between 17–30% [146, 148, 155, 156] and are usually higher than in patients suffering from confirmed asbestosis. See Table 2.

In patients with asbestosis, the data in the literature about a neutrophilic alveolitis are more conflicting, since the mean values reported so far vary considerably, see table 2 [35, 146, 151, 154, 157–159].

Different forms of occupational exposure and different types of asbestos fibres may explain these discrepancies, and future studies should address this topic.

Silicotic disorders. In the silicotic group of patients, data in the literature seem to be more consistent. In coal workers pneumoconiosis a normal percentage of lymphocytes and a mild increase in neutrophils has been reported [151, 152]. In other forms of silica exposure or disease, mainly mixed dust pneumoconiosis, a moderate increase in lymphocytes, sometimes also in neutrophils, has been described [49, 146, 147, 149–151].

Hard metal lung disease. In hard metal lung disease also, the percentage of lymphocytes may be mildly increased [160, 161]. Others have reported an increase in neutrophils and/or eosinophils [162, 163]. An additional characteristic feature of this disease is the presence of increased numbers of giant cells in BAL fluid [162, 163].

Chronic beryllium disease. Chronic beryllium disease is a granulomatous lung disorder that is histologically and clinically identical to sarcoidosis. The BAL cytology shows the same profile as active sarcoidosis with a marked increase in lymphocytes that bear the phenotype of activated T-helper cells namely the CD4+HLA-DR+ phenotype [164–167].

The main difference to sarcoidosis is that the antigen is known in chronic beryllium disease. This fact can be used for a specific diagnostic *in vitro* test measuring the proliferative response to beryllium salts of blood or BAL lymphocytes. In this lymphocyte transformation test, the specific response is almost entirely confined to the CD4+ T-cell subset [167], and is significantly greater from BAL than from blood cells [164, 166, 168]. The blood cell response does not clearly separate patients with chronic beryllium disease from normal controls or from patients with sarcoidosis, whereas with BAL cells the sensitivity of this test has been reported to be 100% in 14 patients with definite chronic beryllium disease, and also the specificity was found to be 100%, indicating that chronic beryllium disease can specifically be diagnosed by a positive proliferative response of BAL cells to beryllium salts [145].

Lymphocyte subpopulations

For asbestosis or asbestos exposure, several groups confirmed that the CD4/CD8 ratio is elevated in some individuals [146, 148, 160, 169, 170]. Only one group reported a decrease in the CD4/CD8 ratio in asbestosis [157]. There are reports indicating that the CD4/CD8 ratio is greater in those with pleural plaques [148, 170]. This relationship was not found in another study, however [146]. The most marked increase in the CD4/CD8 ratio occurs in chronic beryllium disease [166]. A decrease in the CD4/CD8 ratio has been described in silicotic disease [146, 147, 149, 169] and in hard metal lung disease [160, 161].

Detection and quantification of dust particles and fibres

The different methods for identification of particles and fibres in BAL have been extensively reviewed in the previous report of this task group on the technical aspects of BAL [1]. The detection of particles characteristic enough for a certain exposure is already possible by routine light microscopy screening. The formation of ferruginous bodies occurs after inhalation of dusts of various kinds. Most frequently such bodies present true asbestos bodies when they are regularly shaped and regularly segmented with a fine central fibre almost invisible by the light microscope [171]. Other fibres that are thicker or irregularly shaped lead to the formation of pseudo-ferruginous bodies, including talc, glass fibres, and coal dust particles [172, 173].

The presence of dust particles in the cytoplasm of alveolar macrophages may suggest exposure to crystalline and metallic particles including silica [49], coal dust, hard metal [162, 172], antimony [174], aluminium [175], iron-rich particles, and alloys used in dentistry [176].

The exact analysis of the chemical composition of the particles can be done by electron microscopy making use of energy dispersive X-ray analysis (EDAX). From this, conclusions regarding the mineral composition of the particles can be drawn [49, 172, 177]. Quantification of the alveolar dust burden has been performed by EDAX microanalysis in silica exposed subjects and shown to be significantly higher than in unexposed controls, but there was no difference between subjects with silicosis and those with exposure only and no disease, regarding the total amount of silica in the BAL samples [178]. Another method is the neutron

activation analysis, which is especially useful for the detection of trace metals in the cell-free BAL fluid, showing high concentrations of tungsten (W), tantalum (Ta) and cobalt (Co) in hard metal lung disease [163].

The quantification of asbestos bodies is best done by filtration of 5–15 ml fresh BAL fluid, cells included, onto millipore filters, and counting the number of asbestos bodies [179]. Uncoated asbestos fibres can only be counted by electron microscopy [177], but this is, so far, without clinical value.

Asbestos body counts correlate with the type of asbestos related disorder being higher in those with benign pleural disease or malignant mesothelioma [179]. Asbestos body counts in BAL correlate closely with concentrations of asbestos bodies in lung tissue obtained by biopsy or at autopsy. A BAL count of more than one asbestos body per ml is highly indicative of a lung concentration exceeding 1,000 asbestos bodies per g dry tissue [180, 181]. Only seven percent of non-asbestos exposed white collar workers have asbestos bodies at concentrations $>1 \cdot ml^{-1}$ BAL fluid [179]. In general, demonstration of dust in the lungs is an indication of exposure but is no evidence of disease. On the other hand, a minority of patients with definite asbestos exposure and disease may have no detectable asbestos bodies in their BAL fluid [179]. Demonstration of dust in BAL is especially useful in patients with ILD or pleural disease who have previously unknown or uncertain exposure to asbestos or other dusts.

Value of BAL for clinical diagnosis and management

The demonstration of dust in BAL fluid or cells is indicative for exposure, but is no evidence of disease. There is currently no known BAL level of particles above which development of disease is inevitable. ILD has to be proven by routine clinical methods like chest radiography, computerized tomographic (CT) scanning and lung function test.

There is no clinical value of differential cell counts in ILD due to occupational dust exposure, except for chronic beryllium disease.

For the management of patients with known ILD due to dust exposure, BAL is currently of no proven value, except for chronic beryllium disease and for the recognition of the co-existence of another disorder of different cause, such as sarcoidosis, hypersensitivity pneumonitis, haemorrhage syndrome and others [182].

The clinical role of BAL in pulmonary histiocytosis X

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Pulmonary histiocytosis X (PHX) is a rare chronic granulomatous disorder involving cells of the monophagocytic system. The diagnostic feature of this disease is the finding of Langerhans cells (LC) which react with the monoclonal antibody CD1 (OKT6) and which contain characteristic cytoplasmic organelles [183, 184]. After its introduction as a new means of studying peripheral lung and alveolar cell populations, BAL has rapidly proved useful in the diagnosis of PHX [185].

Diagnostic value of BAL in PHX

Several studies have shown the major value of BAL in the diagnosis of PHX [185, 186]. The total cell count is usually increased. HANCE *et al.* have reported that 90% of their PHX patients were smokers [186]. It is well known that the total cell recovery is usually higher in smokers than in nonsmokers. Besides, the nonsmoking patients with PHX have a normal alveolar cell count. The differential cell count shows a high percentage of alveolar macrophages (AM), a slight increase of neutrophils and eosinophils [185]. On electron microscopy, a significant percentage of Langerhans cells (LC) display highly specific pentalaminar structures of constant width, with a tennis racket shape at one end [183, 185]. As this ultrastructural analysis is time consuming, a more rapid and sensitive technique has been developed using monoclonal antibodies to LC (CD1 positive cells) [184]. For some other authors, the finding of PS 100 BAL positive cells could ensure the diagnosis of PHX. However, this antibody is far less specific of LC than CD1 and its use is therefore not recommended.

The actual value of BAL and in particular the presence of LC in the diagnosis of PHX is difficult to assess. Some authors have reported a mean of 5% CD1 positive cells in the BAL of patients with PHX, while in other interstitial lung diseases, less than 3% of the total cells were found to be CD1 positive [184].

In fact, recent studies have shown that LC are normally present in the lower respiratory tract and in lung parenchyma of normal subjects, particularly in smokers [186, 187]. Alteration of this epithelium seems to be an important stimulus in attracting LC to the lung [130], and cigarette smoking is known to produce such epithelial abnormalities in the lower respiratory tract. Besides, cigarette smoke actually increases the number of LC found in BAL fluid [186].

Furthermore, LC have been found in the lung of patients with diseases other than PHX, in fibrotic lung disorders, benign inflammatory conditions or bronchoalveolar carcinoma for instance [65, 167, 168]. Therefore, as the mere presence of LC in BAL is not pathognomonic of PHX, particularly in smoking patients, a percentage of at least 5% of CD1 labelled alveolar cells is required to confirm the diagnosis.