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Clinical guidelines and indications for bronchoalveolar lavage (BAL): Report of the European Society of Pneumology Task Group on BAL

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Contents

Introduction

As for many clinical tools, there is at the present time no clear agreement on the appropriate clinical use of BAL. Undoubtedly, the recent and encouraging clinical experiences with BAL for diagnosis of opportunistic infections in the immunocompromised patient have encouraged a universal acceptance and interest in BAL. Because of the low morbidity of the lavage procedure and the significant yield of clinically important information, many physicians have been encouraged to perform a lavage during bronchoscopies undertaken for a variety of indications. This has resulted in a considerable body of experience with BAL in a number of clinical settings. For many years, one of the main obstacles for general acceptance of BAL as a clinical tool has been the vast disparity among centres worldwide regarding the technique and the processing of the BAL material.

In order to address this important issue of standardization the European Society of Pneumology (SEP), in 1988, set up a Task Force on Bronchoalveolar Lavage. The first report of the group focused specifically on technical recommendations and guidelines on how to perform BAL and how to process BAL material and was published in 1989 in this journal [1].

This is the second joint report of the SEP Task Group on BAL and gives appropriate guidelines and information about the clinical indications and use of BAL in various diseases of the lung. The members of the Task Group have collected all relevant information so far available about the clinical usefulness and indications of BAL. As a result of a critical review of the material and with the help of two consensus conferences of the group this state-of-the-art paper has been produced. It was the aim of the group to provide a short and informative report for the use of clinicians. Thus, this report is not intended as a comprehensive review of each of the topics. It provides guidelines and recommendations about the clinical value of BAL for diagnosis, for prediction of prognosis, and gives some comparative evaluation of BAL to other established investigative means. Only the pertinent literature for these issues is referenced. A small chapter deals with therapeutic applications of bronchial lavage or bronchoalveolar lavage.

Because the field of BAL worldwide is so rapidly evolving and the application of BAL is so widespread, this report can only give recommendations and guidelines, and should not be regarded as an "indication book". There will be several centres where a special expertise for diagnostic applications of BAL has been accomplished which will regard our recommendations as being too restrictive; and there will be other centres also which are still in a learning and experimental stage regarding the clinical use and performance of BAL. Therefore, our Task Group tried to meet the understanding and the requirements of most of the centres currently performing BAL and to give a fair balance regarding our clinical recommendations.

H. Klech

Side-effects and safety of BAL

H. Klech and C. Hutter

Today, BAL is regarded as a very safe procedure. Side- effects are more or less comparable to regular fibrebronchoscopy unless specific invasive procedures like transbronchial lung biopsy are performed. The overall complication rate with BAL is reported to be 0–3% in comparison to 7% with transbronchial lung biopsy and 13% when using open lung biopsy [2]. So far no lethal complication directly attributable to BAL has been reported. Lethality for transbronchial biopsy is reported to be 0.2% and for open lung biopsy 1.8% [2].

Minor side-effects of BAL include coughing during lavage, fever and chills some hours after lavage (which can usually be treated with the help of simple antipyretics), transient alveolar infiltration in the dependent lung segment 24 h after the procedure, transient deterioration of lung function parameters like vital capacity, forced expiratory volume in one second (FEV_1), decrease of oxygen tension (Po_2) (consequences of saline lavage are expressed more in patients with underlying pulmonary diseases in comparison to healthy volunteers). Most side-effects reported are closely related to endoscopic technique, location and extent of lavaged lung area, volume and temperature of instilled fluid (summary in table 1).

Supplemental oxygen delivery as well as ear oximetry and electrocardiogram (ECG) monitoring is strongly advised in patients with severe underlying diseases or in any other critical condition [3]. Patients with mild asthma have been successfully lavaged [4], however, patients with a history of asthma bronchiale should be handled with special caution and careful monitoring is advised [5, 6]:

1) Supplemental oxygen with a nasal prong should be administered throughout the entire procedure.
2) Premedication with aerosolized beta-agonists.
3) Ear-oximetry and ECG-monitoring.

Table 1. – Consequences and side effects of BAL

Alveolar infiltration	<10% of cases, usually subside after 48 hours[§]	7, 8, 9
Crackles	withing 24 hours over dependent areas[§§]	5, 10
Wheezing	in hyperreactive patients up to 1–2 weeks	4
Bronchospasm	rarely in normoreactive, more frequent in hyperreactive patients	4, 5, 9
Fever	10–30%, some hours after BAL[§§]	7, 8, 11, 12, 14
Lung function	transient decrease of FEV_1, VC, PEF, Po_2[§§, $, $$]	5, 11, 12, 13, 14, 15, 16, 17, 18
	transient rise of Pco_2 in patients with COPD	19
Bronchial Reactivity	no change after BAL	15, 20
Epithelial integrity	no effect on lung epithelial permeability 24 hours after BAL	21
	transient decrease of ciliary beat frequency	2
Bleeding	insignificant	9

§: Risk increases with size of instilled lavage fluid volume and numbers of lavaged segments; §§: Risk increases with volume of instilled lavage volume; $: More likely in hyperreactive patients or in patients with severe underlying infiltrative lung diseases; $$: Supplemental oxgyen prevents hypoxemia during BAL.

The clinical role of BAL in idiopathic pulmonary fibrosis

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The aim of this paper is to review the literature on the clinical value of bronchoalveolar lavage (BAL) in the diagnosis and management of patients with idiopathic pulmonary fibrosis (IPF) (synonym: cryptogenic fibrosing alveolitis). This topic has been included in a number of recent detailed reviews [22–24]]. IPF is one of the most serious interstitial lung diseases. The prognosis is poor, with a mean survival of only 3–5.6 yrs [25–27], but progression is very variable in individual patients. Objective response to corticosteroids is achieved in only about 20% of cases [25, 26, 28], and prognostic factors associated with favourable response are younger age, shorter duration of disease [27–29], and more cellular lung biopsies [26, 30, 31]. Thus, it is important to achieve diagnosis and start treatment as soon as possible.

Diagnostic value of BAL in IPF

There are no specific diagnostic BAL features in IPF, but useful information can be provided by the differential counts of BAL cells, and the profile of BAL cell types. Different types of increased BAL cells predominate in the different interstitial lung diseases, which do not provide a definitive diagnosis because of variation within, and overlap between, disorders but trends of difference between the disorders can support the provisional diagnosis or suggest an alternative.

Neutrophils are the main lavage cell type increased in IPF [32–34] and in other diffuse interstitial fibrosing lung disorders including fibrosing alveolitis associated with collagen vascular diseases (see below), the inorganic dust disease asbestosis [35], and experimental models of silicosis [36]. Patients with IPF, collagen vascular diseases, and asbestosis also frequently have increased eosinophils in lavage [34–38]. Apart from this, high counts of eosinophils in lavage have only been reported in cases of eosinophilic pneumonia, in patients with Churg-Strauss syndrome and in patients with in asthma [39].

The most useful aid to diagnosis is given by the full profile of BAL cell types increased in each patient. The combination of increased neutrophils and eosinophils occurs in about two-thirds of patients with

IPF [34, 40] and in asbestosis [35], but is very rare in patients with granulomatous lung diseases where lymphocytes are the predominant increased BAL cell type. Furthermore, the distinction between IPF and asbestosis is aided by the identification of asbestos bodies amongst the lavage cells, which indicate that exposure has taken place and that the diagnosis of occupational lung disease must be considered [25, 35, 41]. Lone neutrophil increases occur in many patients with IPF but caution must be taken regarding the diagnostic interpretation, since moderate increases can arise for many reasons, and very high counts occurring alone can suggest bacterial infection. However, it is of interest that neutrophil counts increase and lymphocyte counts tend to fall as the grade of radiographic shadowing and fibrosis increases in patients with sarcoidosis [42–44].

A minority of IPF patients show a less typical BAL cell profile. In particular, the subset who respond favourably to corticosteroids frequently have slight to moderate increases in BAL lymphocytes in association with neutrophils but very rarely with eosinophils [34, 45–47]. Increases in BAL lymphocytes have also been reported in workers exposed to asbestos or silica at a stage prior to the development of symptoms [48, 49].

Increased T-helper/suppressor BAL lymphocyte ratios have recently been reported in IPF, contrasting with reduced ratios in patients with associated collagen vascular diseases [50, 516], but the diagnostic value of this approach is restricted since increases in BAL lymphocytes are relatively infrequent in these diseases. Measurement of carcinoembryonic antigen in BAL fluid has recently been claimed to be a possible marker of early malignant change in the clinical course of IPF [52]. Physicians should also be aware that alveolar lipoproteinosis can very occasionally develop in patients with IPF following treatment with corticosteroids [53]. It is also important to be aware that findings similar to those in patients with IPF have recently been reported in clinically unaffected family members, namely increased numbers of neutrophils, evidence of macrophage activation, and growth factors for lung fibroblasts [54].

In conclusion, inclusion of lavage in the pre-treatment investigation of patients with IPF, although it is not pathognomonic, can give some support to the diagnosis, when considered in the full clinical context. However, once patients have commenced therapy this can influence the lavage findings (see below).

Prognostic value of BAL in IPF

Pre-treatment BAL cell counts may be of some value in the clinical management of IPF patients as a prognostic indicator of response to therapy.

Patients with increased percentage counts of BAL lymphocytes have a significantly better chance of responding to corticosteroids than the remainder [34, 45–47]. By contrast, percentages of neutrophils and eosinophils are significantly higher in those who fail to respond to steroids [34, 45, 55] and patients with increased eosinophils have an especially poor response [34, 40, 45, 46, 56, 57]. However, there is a recent report that some patients with increased eosinophils can respond to cyclophosphamide (100 mg per day) combined with prednisolone (20 mg per alternate day) [58]. It is hoped that future prospective trials may show that pre-treatment lavage cell counts may be of value to indicate the most appropriate drug for the individual patient.

Numerous other markers can be measured in BAL samples, but there is little information on their correlations with clinical features. It has recently been reported that IPF patients with high concentrations of myeloperoxidase [59], and those with higher levels of hyaluronate and type III procollagen peptide [60] in BAL fluid deteriorate more rapidly than those with low levels; that patients with increased histamine in BAL fluid have higher grades of fibrosis in their lung biopsies [41]; and that patients with late stage IPF have low levels of proteolytic activity in the BAL fluids [62]. Factors released from activated alveolar macrophages may play the major role in stimulating the growth of fibroblasts in IPF [63], but the clinical value of measuring such markers is unknown. However, since colchicine can suppress the production of these factors *in vitro*, it has been suggested that this drug may have a potential role in the treatment of IPF [64].

In conclusion, the current evidence on the prognostic value of lavage findings in IPF suggests that the information may be of some value in guiding the selection of therapeutic agents.

The value of BAL in monitoring and surveillance of therapy in IPF

The safety of BAL makes it an ideal technique to monitor changes occurring with disease progression and under the influence of therapy, but there is still relatively little information on serial lavage studies in patients with IPF. One series of patients has been followed from 1–7 yrs, mean 4 yrs [58]. Patients responding to high dose prednisolone showed a significant fall in the percentages of all inflammatory cell types, but most notably in neutrophils, while counts remained elevated or increased in the non-responders; patients followed on treatment with cyclophosphamide plus low dose prednisolone, showed a significant fall in eosinophils in the responders, but not in the non-responders. Another study has also found that corticosteroid treatment does not suppress BAL neutrophils in non-responders after 3 mths or 6 mths of therapy, but stated that patients failing to respond to cyclophosphamide alone or plus corticosteroids showed a significant reduction in neutrophils at 3 mths and at 6 mths [65]. By contrast, a third study has observed that BAL neutrophil counts increased after 3 mths prednisolone in smokers, but not in nonsmokers, with IPF who showed clinical improvement [66]. However, the follow-up periods were very different in the three studies, up to 7

yrs in the former [58], but only 3 mths [65, 66] and 6 mths [65] in the latter. Thus, it is still premature to draw conclusions on the clinical value of lavage cell counts in monitoring the progress of IPF patients.

Serial lavage studies have also recently shown that proportions of phosphatidylglycerol, which are reduced in the BAL fluids of many untreated IPF patients [67, 68], return to normal in patients responding to prednisolone but not in non-responders [67]. It has been suggested that such changes may reflect the extent of damage to the alveolar epithelium in IPF.

In conclusion, preliminary reports indicate that BAL may be of clinical value to monitor changes in the lungs associated with therapeutic response in IPF, but further information is required. In particular, independent prospective studies are needed where patients are evaluated over comparable long-term periods, and details are required of survival as well as radiological and functional response to therapy.

Conclusions

Current published evidence suggests that lavage is of value to aid the diagnosis and management of patients with IPF. BAL cell counts are only a guide to the differential diagnosis of IPF because of the variability within and overlap between diseases. Nevertheless, BAL is of particular value to identify and exclude some of the rarer lung diseases which must be considered in the provisional diagnosis. BAL can provide some useful prognostic indicators in IPF which may aid therapeutic decisions, and serial BAL measurements may have a place in assessing suppression of inflammation in patients responding to therapy. However, at this stage in our knowledge caution should be given to the interpretation of BAL findings, and they are most useful when considered and interpreted in the context of the overall clinical and other investigatory techniques used in the diagnosis and management of patients with this serious lung disease.

Collagen-vascular diseases

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Inflammatory processes that develop in the lung in many of the collagen vascular diseases (CVD) usually result in a diffuse interstitial lung disease (ILD) similar to idiopathic pulmonary fibrosis. Chronic alveolitis, as assessed by bronchoalveolar lavage, revealed the same characteristic pattern of alveolar inflammation associated with idiopathic pulmonary fibrosis; which is evidence of neutrophil accumulation and macrophage activation [38, 45, 50, 69–85]. However, there is a considerable overlap for each disease and type of alveolitis. In addition, inflammatory alveolitis may also be present in a high proportion of patients with CVD and without clinical or radiological evidence of pulmonary involvement, suggesting the presence of an ongoing subclinical alveolitis.

Cellular characteristics of alveolitis

Total number of recovered cells is increased in patients with overt ILD but not in patients without ILD. In addition, total number of cells is progressively reduced in advanced progressive systemic sclerosis [77]. The distribution of BAL cell type according to the disease and to the presence of an associated ILD is summarized in table 1. In addition, alveolar macrophages are "spontaneously" activated and release various bioactive mediators that could be relevant to the pathogenesis of ILD: superoxide anion (various CVD), neutrophil chemotactic factors (various CVD), fibronectin (various CVD), alveolar macrophage derived growth factor for fibrosis (AMDGF) (progressive systemic sclerosis) and tumour necrosis factor (TNF) (rheumatoid arthritis).

It appears that symptomless patients with CVD can have a similar pattern of alveolar inflammation including accumulation of neutrophils and/or lymphocytes and activated alveolar macrophages [86–91].

On the other hand, some cell activities may be defective: since decreased antibacterial activity of alveolar macrophages has been reported in systemic lupus erythematosus but not in other CVD [92, 93].

Biochemical characteristics of alveolitis

The biochemical analysis of BAL fluid shows an increased transudation of serum factors and/or an increased secretion of mediators: albumin, immunoglobulin G (IgG), IgM, alpha-2 macroglobulin, plasminogen activator, procollagen peptide (progressive systemic sclerosis), collagenase, elastase [73, 76, 83, 84, 94, 95]. So far, the value of biochemical analysis of BAL fluid in diagnosis and management of ILD CVD remains to be established.

Clinical significance of alveolitis in CVD

Since alveolar inflammation is a characteristic feature of CVD patients with or without associated ILD, the BAL cytology is by no means a reliable argument for the diagnosis of ILD in this context. However, BAL may be useful for the diagnosis of an associated

Table 1. – BAL cytology in collagen vascular diseases

Disease	With ILD	Without ILD	Ref.
Progressive systemic sclerosis	neutrophils eosinophils	neutrophils eosinophils	[38, 70–72, 74, 75, 86]
Rheumatoid arthritis	neutrophils lymphocytes	lymphocytes (CD4+, T5/9)	[76, 79, 83, 85, 86, 90] [78]
Primary Sjögren syndrome	neutrophils, lymphocytes (CD8+)	lymphocytes (CD4+)	[86–88, 100]
Systemic lupus erythematosus	neutrophils, lymphocytes	lymphocytes	[92]
Dermatopolymyositis	neutrophils	neutrophils	[86, 99]
Mixed connective tissue disease	neutrophils	neutrophils	[86]
Secondary Sjögren syndrome	neutrophils, lymphocytes (CD8+)	neutrophils, lymphocytes (CD8+)	[86, 88]

Presence of ILD is judged by clinical and radiological findings.

lung disease (infection, pulmonary haemorrhage, alveolar proteinosis *etc.*) or of drug-induced lung disorder [96–98]. BAL may also be useful to assess the activity of acute or chronic ILD in patients with scleroderma or with dermatopolymyositis [72, 82, 99]. In general, when increased numbers of lymphocytes are present in BAL fluid, lung disease is associated with a relatively good prognosis, whereas the presence of a predominantly neutrophilic or eosinophilic alveolitis is associated with a higher risk of functional and radiographic deterioration.

The role of BAL in therapeutic decision in symptomless patients with CVD is unclear since its prognostic value is still controversial. Preliminary data suggest that: 1) lymphocyte alveolitis is of good prognosis; 2) neutrophil alveolitis is associated with progressive deterioration of pulmonary function test (PFT) over a 1 yr follow-up in untreated patients. However, corticosteroid treated patients can improve their PFT while the alveolar neutrophilia persists.

In summary:

1) BAL may be useful for the diagnosis of lung complications in CVD; there is as yet no convincing evidence that BAL provides any help in the diagnosis and the management of chronic ILD-CVD.

2) BAL may be useful in the clinical management of acute ILD-CVD .

The value of bronchoalveolar lavage in the diagnosis and prognosis of sarcoidosis

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There is a consensus that BAL changes in sarcoidosis reflect the pathological process [101–110]. Furthermore by analysis of CD4/CD8 lymphocytes BAL can be of benefit in distinguishing sarcoidosis from other granulomatous diseases, such as hypersensitivity pneumonitis [111, 112]. Whether BAL can be used diagnostically and/or prognostically depends, however, on two factors. Firstly, for lavage analysis to be diagnostic, features have to be recorded that together with clinical investigation represent a unique picture of this disease and discriminate it from other interstitial lung diseases. Secondly, there has to be a clear clinical understanding of the level of disease "activity" for the features identified in lavage to be measured against. This second condition is somewhat difficult to satisfy as there appears to be no easy clinical measure of activity. It is only when patients have advanced to fibrotic forms of disease that clear clinical reflections of disease outcome are observed. The values of BAL to diagnosis and prognosis are commented on in tables 1 and 2. Emphasis on the prognostic value of a mere increase of the BAL lymphocyte count, interpreted as high intensity alveolitis [109] weakened as it was made obvious that even advanced cases may show a normal BAL lymphocyte count [113, 114]. BAL lymphocyte counts appear too unreliable as a single investigative tool to be of help regarding therapeutic decisions in patients receiving corticosteroid treatment [115].

A characteristic pattern of BAL macrophage phenotypes identified by monoclonal antibodies

(RFD7+/RFD1-) have been described being predictive for prognosis [116, 117]. However, since those monoclonal antibodies so far are not commercially available, their use has not been included in our clinical recommendations.

Cells and soluble factors not mentioned in these tables have not as yet been investigated sufficiently to make any comment.

Conclusions

No single feature in BAL is diagnostic of sarcoidosis. The combination of parameters listed below would be consistent with sarcoidosis in an appropriate clinical setting:

1. Lymphocytosis;
2. CD4:CD8 ratio >3.5;

Biochemical profiles of lavage constituents might be of value if reliable and reproducible methods can be found to measure the *in situ* concentrations.

The prospect of using lavage analysis to determine prognosis is promising but standardization of lavage method and better clinical definitions of disease activity are required before this could be routinely used. There are, however, features in BAL that are associated with progression to fibrosis.

Table 1. – Lavage factors in the diagnosis of sarcoidosis

Substance/cells	Comment	Clinical value	Ref.
A.C.E.	Raised in >60% of cases	Not established	[118]
Procollagen III peptide	Raised in many cases but not specific for sarcoidosis	Not established	[119]
Hyaluronic acid	Raised in many cases but not specific for sarcoidosis	Not established	[120]
T-cells	T-cell lymphocytosis is a consistent BAL feature in sarcoidosis although odd cases may show normal lymphocyte proportions	Helpful in the appropriate clinical setting	[103, 109, 110, 121]
CD4:CD8	Raised ratios in the presence of lymphocytosis show high specificity for sarcoid	Possible adjunct to diagnosis	[121]
$01^+/07^+$ macrophages	Cousistently >20% in sarcoidosis, but reagents not yet commerically available	Helpful if combined with other parameters	[116]

Table 2. – Lavage factors in the prognosis of sarcoidosis

Cell type*	Comment	Ref.
T-lymphocyte	Too variable to act as a prognostic indicator. Advanced cases can show no increase in lymphocytes	[114]
CD4:CD8 ratio	Some correlation has been shown between raised ratio and poor prognosis. Patients with high ratios should be followed closely	[122, 123]
Neutrophils	Raised neutrophils may indicate move to fibrosis. Not specific for sarcoidosis but may be of value in appropriate clinical setting	[43]
Mast cells	There is some evidence that raised mast cells in BAL is a prognostic sign. These cells may be involved in the initiation of fibrosis. Technical difficulties in identifying mast cells (*i.e.* appropriate fixation) should be noted	[124, 125]

*: no soluble factors in lavage have been shown to be of prognostic value.

Extrinsic allergic alveolitis

G. Semenzato, L. Bjermer, U. Costabel, P.L. Haslam, D. Olivieri

Extrinsic allergic alveolitis (EAA) or hypersensitivity pneumonitis is an interstitial lung disease associated with repeated exposure to a wide range of inhaled organic dusts and related occupational allergens, especially bird and fungal proteins. We focus on the diagnostic and prognostic findings related to the use of BAL in the management of EAA patients.

The presentation of EAA varies from patient to patient and is mainly related to the frequency and intensity of exposure to the causative antigens. In addition, the different amounts of inhaled antigens and the timing of observation might be crucial. For this reason we will refer to the acute, subacute or chronic phases of the clinical picture rather than to the active and inactive forms of the disease.

Analysis of cellular constituents of BAL

As far as the evaluation of cellular constituents of BAL of patients with EAA performed more than one week after the acute episode is concerned, cellular recovery is approximately fivefold that observed in controls, with the cells accounting for this increase mostly represented by lymphocytes [112, 126, 127]. The increase of lymphocytes becomes evident several days after the acute episode and represents the most striking finding during the entire follow-up of the disease process.

Some authors focused their attention on the importance of an additional mild increase of neutrophils in the BAL of EAA patients [22, 128]. The number of neutrophils has been proved to be markedly increased in the acute phase of the disease, soon after the exposure to specific antigen or after the challenge with EAA causing antigens. This effect was only short-term since one week after challenge the neutrophils had fallen to pre-challenge values [128].

In the early phase of the disease mast cells have also been reported to increase in number [108–110]. This increase seems to be correlated to the phase of exposure, with a more than one hundredfold increase in the acute disease, and during recovery declining towards normal within a few months [128, 132]. A few plasma cells (0.1–2%) have also been observed in subacute stages of EAA [133].

Evaluation with monoclonal antibodies revealed that the most common finding in EAA patients is represented by the expansion of lymphocytes bearing the cytotoxic/suppressor phenotype. In fact, immunological evaluation of surface markers demonstrated that only a few BAL lymphocytes express B-cell related determinants, the majority of them being represented by T-lymphocytes [112, 126, 127].

The analysis of T-cell subsets revealed that in the majority of cases CD8+ lymphocytes are the predominant cells in the BAL of these patients. As a result, the CD4/CD8 ratio is low (usually less than 1.0). The number of cells bearing the proliferation associated markers (CD71 and CD25 antigens) is quite low; a statistically significant difference with respect to controls exists regarding their absolute numbers [127]. Also, the percentage and absolute number of T-cells expressing HLA-DR antigens is increased [134].

With regard to the frequency of cells bearing NK- related markers, notably the pattern of reactivity with MoAbs defining natural killer cells, only the positivity for CD57 MoAb is increased significantly with respect to controls [126, 117, 135]. The frequency of CD57 cells co-expressing T-cell markers is predominant over the number of cells that lack these antigens [117, 135].

Thus, the alveolitis in EAA patients is characteristically represented by cells with the CD3+/CD8+/CD57+/CD16- phenotype. In fact, at the present time, this phenotype has not been observed in other conditions, including HIV infection [136].

So far, phenotyping of alveolar macrophages cannot be recommended for the clinical use of BAL.

Diagnostic relevance of BAL findings in EAA

At present, we can only state that BAL can assist in achieving the diagnosis of EAA. Interestingly, in this disorder you can find the highest degrees of BAL lymphocytosis (an even higher average than in sarcoidosis). Therefore, the presence of a marked lymphocytosis characterized by the CD3+/CD8+/CD57+/CD16- phenotype is highly suggestive of EAA. In fact, no cases have been reported with normal BAL cytology. A "positive" BAL finding (*i.e.*, the characteristic profile) in a patient with interstitial lung disease of unknown origin should direct the clinician towards the probable diagnosis of EAA. A careful re-examination of the occupational environmental history and the screening of serum precipitins might then reveal previously unknown sources of relevant antigen exposure and confirm the diagnosis of EAA.

From the clinical point of view, BAL has the advantage of being the most sensitive tool in detecting signs of alveolitis in EAA patients, more sensitive than chest radiography, lung function testing and precipitins. We must, however, be careful to exclude, by history or other clinical tests, the disorders that are also characterized by an infiltrate bearing the suppressor/cytotoxic phenotype, including patients with interstitial pneumonia associated with collagen vascular disease, silicosis, bronchiolitis obliterans with organizing pneumonitis

(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

C. Danel, D. Israel-Biet, U. Costabel, G.A. Rossi, B. Wallaert

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.

On the other hand, with PHX having a patchy distribution, a localized BAL can miss the diagnosis, as well as a transbronchial biopsy. Confirmation by an open lung biopsy is therefore advisable.

Conclusions

There are strong arguments to support the usefulness of lavage cell analysis in the diagnosis of pulmonary histiocytosis X. However, false negative results can be related to the patchy distribution or to the stage of the disease. False positive results havealso been reported in heavy smokers or in bronchoalveolar carcinomas, for instance. This highlights the fact that BAL data should be interpreted carefully in the context of clinical and radiological data. One requires at least 5% of LC in BAL to confirm the diagnosis. This either gives sufficient diagnostic clues or else points to the necessity of an open lung biopsy.

The clinical role of BAL in eosinophilic lung diseases

C. Danel, D. Israel-Biet, U. Costabel, G.A. Rossi, B. Wallaert

Eosinophilic infiltrates in the lung can be encountered in a great variety of disorders such as asthma, eosinophilic pneumonia, allergic bronchopulmonary aspergillosis or Churg and Strauss vasculitis. In this chapter we will concentrate on eosinophilic pneumonia ranging from the acute but mild and remitting Loeffler's syndrome to the severe chronic eosinophilic pneumonia. As these diseases can be life-threatening but remarkably reversible under corticosteroid therapy, a rapid diagnosis is of major importance. Since no alveolar eosinophilia is ever observed in normal controls, any increase in the percentage of eosinophils in BAL argues for a pathological process. In any type of eosinophilic lung (EL), acute or chronic, BAL always displays a high alveolar eosinophilia, whether or not associated with a blood eosinophilia [190–193].

Besides its diagnostic value, BAL has also given clues to the pathogenesis of eosinophilic lung injury. Indeed, eosinophils secrete not only neutral proteases and oxygen radicals but also a major basic protein (MBP) and a cationic protein (ECP) known to be able to induce acute lung damage and pulmonary fibrosis [194]. Finally, BAL is also valuable in EL for the clinical follow-up of patients under treatment [195].

Diagnostic value of BAL in eosinophilic lung

As, in these disorders, eosinophils are largely located in air spaces, the diagnostic yield of BAL is very high, usually making more invasive techniques (open lung biopsy or transbronchial biopsy) unnecessary. The analysis of BAL and blood should be performed in parallel. The diagnostic value of a high alveolar eosinophilia is all the greater if the level of the blood eosinophilia is normal.

It is usually in eosinophilic pneumonia (EP) that the highest eosinophilic count is observed [190–193]. If the increase of total recovered cells is not always significant, the percentage of eosinophils is markedly abnormal, sometimes increased up to 90% of total cells, associated or not to a few mast cells, and always higher than the neutrophil count. A proportion of these eosinophils can undergo necrosis, and fine eosinophilic granules can be observed in alveolar macrophages. Nevertheless, such a high alveolar eosinophilia can also be observed in some parasitic disorders or in the Churg-Strauss syndrome [192]. Less pronounced eosinophil increases (5–10%) can be found in sarcoidosis, histiocytosis X, drug induced pneumonia, collagen vascular disease, asthma and idiopathic pulmonary fibrosis [190–192].

Conclusions

In eosinophilic lung diseases (EL), BAL is of great value not only for the diagnosis and the follow-up of patients treated, but also for the study of their pathogenesis. EL is one of the diseases in which BAL can give enough clues to the diagnosis to avoid, in many cases, an open lung biopsy. The highest eosinophil counts ever seen in BAL fluid are observed here, ranging from 20–90% of the cells. These results are most useful when the X-ray findings are atypical and peripheral eosinophilia absent.

The clinical role of BAL in alveolar proteinosis

C. Danel, D. Israel-Biet, U. Costabel, G.A. Rossi, B. Wallaert

Pulmonary alveolar proteinosis (PAP) is a rare disorder characterized by accumulation of periodic-acid-Schiff (PAS)-positive phospholipidic material in the alveolar spaces [196]. PAP can be idiopathic or secondary to various conditions, such as immunosuppression, malignant haematological disorders, silicosis or, more rarely, diffuse interstitial lung diseases [53, 196, 197].

As the clinical and radiological presentations are not specific, PAP can remain misdiagnosed. Segmental BAL appears to be essential in management of this disease for diagnosis, follow-up, and therapeutic purposes [197].

Diagnostic value of BAL in PAP

Several studies have shown the major value of BAL in the diagnosis of PAP [196–198].

On gross examination, the BAL fluid has a milky appearance. After gravity sedimentation a dense tan sediment can also be observed. On light microscopy, the analysis of recovered cells shows an increase in total cell count [199–1009] probably partially explained by the fact that, in these studies, the majority of patients were smokers. On cytocentrifuged slides stained by MGG, the striking feature is the finding of a variable amount of basophilic extracellular deposit mixed with enlarged foamy alveolar macrophages (AM), crystal clefts and cellular debris. This extracellular material as well as the cytoplasmic content of the AM show a pink PAS positive diastase resistant staining. It should be noted that this staining is weaker than that observed in transbronchial biopsy (TBB) or open lung biopsy due to the dilution induced by the BAL fluid.

On electron microscopy the ultrastructural appearance is characteristic, with small lamellar bodies of wavy or regular periodicity, tubular myelin structures and myelin-like multilamellated structures with electron dense central region [101–102]. Added to this extracellular material, ghost cells, AM and/or pneumocytes II are filled with intracellular bodies and empty vacuoles or grey lipid droplets.

Different cellular profiles have been described. Some authors found an increase of lymphocytes compared to a control group with similar smoking habits [200] with an increased ratio of helper to suppressor T-cells. Others found a slight increase in neutrophils [203]. Particularly in these latter cases, a careful search for pathogens has to be undertaken.

In order to differentiate primary from secondary PAP, some authors have proposed an analysis of the alveolar material with specific antibodies against surfactant apoproteins. They have shown a significant difference in the quantity and repartition of the staining between primary and secondary forms [204].

Biochemical analysis of the lavage fluid, in particular protein and lipid analysis, have been performed by many laboratories. In comparison with normal subjects, a higher protein and phospholipid concentration is always present, and qualitative abnormalities in phospholipid composition have been found [53, 205]. Some authors have shown an impairment in AM function [199, 206].

The value of BAL in comparison to other diagnostic procedures in PAP

Few papers have compared the advantages of the different diagnostic procedures in PAP. However, in comparison with sputum analysis, transbronchial biopsy (TBB) or open lung biopsy, they have emphasized the major value of BAL [196, 197, 201, 202]. This is mainly due to the fact that PAP is an intra-alveolar disease and that, for instance, segmental BAL covers a larger distal lung field than TBB, the latter being sometimes equivocal if the disease is patchy. Nevertheless, the combination of both procedures will assure proper diagnosis. However, as TBB can induce alveolar space oedema and focal haemorrhages, BAL should be performed first.

The value of BAL in the follow-up and treatment of PAP is reported in the chapter dealing with therapeutic applications

Conclusions

Compared with other pulmonary disorders, PAP is certainly that in which BAL has a very high diagnostic yield, making open lung biopsy in most cases unnecessary. Furthermore, BAL is also of major value in the follow-up and the therapeutic management of patients with PAP.

The clinical role of BAL in pulmonary haemorrhages

C. Danel, D. Israel-Biet, U. Costabel, G.A. Rossi, B. Wallaert

Many different clinical syndromes are included under the general heading of pulmonary haemorrhages (PH) and haemosiderosis (table 1). The triad of haemoptysis, infiltrates on chest X-ray and anaemia are present in most of the cases, however active PH does occur without these findings.

Furthermore, a delay in diagnosing PH can lead to fatal renal or pulmonary complications. Therefore, a rapid diagnosis is important and BAL appears to be the method of choice especially to diagnose distal occult PH and to eliminate other underlying diseases such as infections or malignancies.

Diagnostic value of BAL in pulmonary haemorrhages

On gross examination, the BAL fluid has either a bloody or orange-pink colour, or can be of normal translucent appearance.

On light microscopy, compared with nonsmoking controls, the total cellular count and the percentage of AM are increased [207]. Several morphological aspects can be observed such as free red blood cells, red blood cells in alveolar macrophages (AM) and haemosiderin laden AM. The importance of the haemosiderin content can be evaluated either by the percentage of AM

Table I – Principal disorders associated with diffuse pulmonary haemorrage (PH) and haemosiderosis

1. PH secondary to cardiac disease, intrapulmonary vascular lesions or malformations.
Chronic left- or right-sided heart failure (mitral stenosis).
Pulmonary hypertension.
Pulmonary veno-occlusive disease.
Pulmonary lymphangiomyomatosis.
Arteriovenous fistulas or other congenital vascular malformations.
Vascular thrombosis with infarction.

2. Pulmonary haemosiderosis and glomerulonephritis.
With anti-basement membrane antibody (ABMA) disease.
Without ABMA.
With immune complex-mediated.

3. Idiopathic pulmonary haemosiderosis.

4. PH associated with vasculitides and collagen vascular disease.
Systemic lupus erythematosus.
Wegener granulomatosis.
Mixed connective tissue disease.
Idiopathic thrombocytopenic purpura.

5. PH associated with miscellaneous disorders.
Diffuse necrotizing infections.
Severe coagulopathy.
Malignant diseases such as acute leukaemia.

6. PH associated with drugs.
D-penicillamine.
Amphotericin B
Chemotherapy drugs

containing haemosiderin or by a score proposed by GOLDE and co-workers [208, 209]. This haemosiderin score (HS) is based on the colour intensity of AM cytoplasm on an iron stain (*i.e.* Prussian blue).

The presence of intact red blood cells in the lavage fluid is not in itself a definite sign in favour of AH, it can be related simply to minor trauma during the bronchoscopy. However, in acute PH such as in Goodpasture's syndrome, BAL can be bloody without haemosiderin laden AM [210]. In fact, rather than a bloody BAL fluid, free red blood cells or red blood cells in AM, it is the presence of numerous haemosiderin laden macrophages, appearing at least 48 h after bleeding, which strongly suggests pulmonary haemorrhage [211]. When one observes not only a large increase in the percentage of AM containing haemosiderin deposits, but also an increase in the intensity of the haemosiderin content (HS >100), the diagnosis of alveolar haemorrhage can be confirmed. In the evaluation of the bleeding, this HS appears more sensitive [207, 212]. In fact, in many pulmonary disorders without significant bleeding, light haemosiderin deposits can be observed, even in a large percentage of AM (such as in immunosuppressed patients).

Comparison of BAL and other diagnostic procedures in PH

Few papers have compared the advantage of the different diagnostic procedures in AH. Compared with transbronchial biopsy (TBB) or open lung biopsy, they have mostly emphasized that BAL is a less invasive technique, particularly important in patients with low platelet counts or bleeding disorders, where biopsy may often be impossible because of the high risk of bleeding [207, 208].

Some authors [207, 212] have compared the haemosiderin score (HS) in BAL [208] and pulmonary parenchyma obtained by TBB, open lung biopsy and from post-mortem specimens. They have shown that in BAL HS was a very good marker of pulmonary haemorrhage. In particular, a high HS is always associated with histological evidence of severe pulmonary haemorrhage. KAHN *et al.* [207] conclude that an HS greater than 100 is indicative of severe pulmonary haemorrhage. On the contrary, there is no correlation between the bloody appearance of the BAL fluid or large number of red blood cells per mm^3 and either an elevated HS or the presence of alveolar haemorrhage in tissue specimens.

Conclusions

BAL appears to be the method of choice to confirm pulmonary bleeding especially in occult alveolar haemorrhages and to search for an underlying disease such as infection or malignancies. It is a safe procedure with minimal and rare complications particularly in patients with low platelet counts or bleeding disorders and can be performed in virtually all cases regardless of the severity of the disease.

Drug induced pneumonitis

C. Danel, D. Israel-Biet, U. Costabel, G.A. Rossi, B. Wallaert

Since the list of drugs that may adversely affect the lung grows longer every day, the problem is not to be exhaustive in naming every one of them but to have reliable criteria by which to suspect and to recognize an iatrogenic lung disease early enough to prevent the development of irreversible injury [213, 214]. In this context, BAL has proved to be a very useful tool in the diagnostic approach. It can provide evidence to differentiate between iatrogenic causes, and to distinguish these from infectious or malignant aetiologies.

In table 1 are listed the main drugs known to be responsible for an iatrogenic lung injury. The pathogenic mechanisms are usually multifactorial.

Table 1. – Main drugs known to be responsible for iatrogenic lung injury

1. Drugs inducing pulmonary haemorrhages

D-penicillamine
Amphotericin B

2. Drugs inducing a lymphocytic/neutrophilic/eosinophilic alveolitis

Lymphocytic	Neutrophilic	Eosinophilic
Methotrexate	Bleomycin	Bleomycin
Azathioprine	Busulphan	Nitrofurantoin
Bleomycin		Cotrimoxazole
Busulphan		Penicillin
Nitrofurantoin		Salazopyrin
Acebutolol		
Gold salts		
Salazopyrin		
Amiodarone		
Propanolol		
Diphenylhydantoin		

3. Drugs inducing a thesaurismosis

Amiodarone
Potentially, all the amphiphilic drugs

Diagnostic value of BAL in drug induced lung diseases

In rare cases, BAL can be sufficient to confirm a suspected diagnosis. The best example is the exogenous lipoid pneumonia induced by mineral oil, taken as nose drops or laxatives. In these cases, alveolar macrophages contain large empty vacuoles representing fatty material strongly stained by the oil red O. Chromatography on thin slices performed on the lipid extract of BAL shows the same physical profile as the drug involved [215].

In some cases of direct toxicity due to drugs such as bleomycin, cyclophosphamide and nitrofurantoin, various forms of pulmonary reactions can be observed, such as diffuse alveolar damage, eosinophilic pneumonia, or secondary alveolar proteinosis. In these cases, BAL will show atypical cells, a high percentage of eosinophils or extracellular lipoproteinaceous debris suggesting a diagnosis of drug induced toxicity.

More frequently, BAL has to be interpreted in the light of other diagnostic information (clinical history and examination findings, radiological features, *etc.*), the cytological profiles encountered here are few and non-specific. Schematically alveolar haemorrhages can be observed, mainly induced by D penicillamine. However, the most frequent BAL feature observed is an alveolitis characterized by an increase in total recovered cells among which one particular cell type can be markedly predominant (lymphocytic alveolitis) [216]. An increase of polymorphonuclear cells and morphological alterations of alveolar macrophages (thesaurismosis) can also be observed [217, 2189]. The hyperlymphocytosis in the context of a drug induced pneumonitis can be as high as 80% of the recovered cells, but usually averages 40–50% [216, 217]. A predominance of suppressor/cytotoxic T-cells of the CD8 type with an inversion of the CD4/CD8 ratio is usually observed [216, 218]. Rarely a predominance of helper T-cells (CD4) is described, such as in methotrexate or nitrofurantoin induced pneumonitis [219, 220]. Associated with the CD8 lymphocytosis, a small proportion of eosinophils, mast cells and basophils is commonly found. Concurrently, although not routinely examined, the BAL fluid composition can be modified in particular with an increase in immunoglobulins [218]. All these features are similar to those found in classical hypersensitivity pneumonitis due to organic antigens. This underlines the fact that such environmental exposures must be excluded before confirming the iatrogenic origin of the lung disease.

An extremely high percentage of unaltered neutrophils usually argues for a very early stage (<48 h) of drug induced hypersensitivity, particularly if a concurrent alveolar haemorrhage is observed [217, 218]. In other cases the percentage of neutrophils averages 10–30%, suggesting the development of a pulmonary fibrosis. This can be due either to a neglected hypersensitivity or to the direct toxicity of drugs such as bleomycin.

Certain drugs, such as amiodarone or more generally any amphiphilic molecule can lead to thesaurismosis. In this disorder, ultrastructural studies of BAL show an accumulation of numerous large lamellar inclusions, phospholipidic in nature, mainly in alveolar macrophages, but also in neutrophils, lymphocytes and bronchial cells [218, 221]. These features have been observed in treated patients whether or not they have developed a pneumonitis. In contrast, hyperlymphocytosis associated with a thesaurismosis has been observed only in treated patients with pneumonitis [211]. Thus, it seems that thesaurismosis is necessary but not sufficient for the development of pneumonitis, which requires in addition an immune mechanism. In these cases BAL alone has no definite diagnostic value but becomes very suggestive in the context of an appropriate clinical presentation.

Conclusions

In drug induced pneumonitis, BAL can show different cellular profiles. None of them is absolutely specific and therefore BAL is not sufficient in itself to give a diagnosis. Nevertheless, it may help in eliminating alveolar haemorrhages, infectious disorders or the recurrence of an underlying disease such as malignancy, which could also be responsible for the pulmonary symptoms. Finally, besides the clinical value of BAL reported above, it should be stressed that it has given several clues to the pathogenic mechanisms of these disorders.

The clinical use of BAL in patients with pulmonary infections

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In immunocompetent patients with community acquired pneumonia, as well as in the immunocompetent host with nosocomial pneumonia, a calculated therapy can be initiated without prior invasive diagnostic procedure. This kind of patient management, however, is not warranted in immunocompromised or immunodeficient patients, in whom an exact diagnosis and the identification of the organism causing pneumonia is of utmost importance to select the correct therapeutic regime. If less invasive techniques like blood cultures were not successful in establishing the diagnosis, or the results from other procedures such as sputum induction were nondiagnostic, it is necessary to obtain specimens from the lower respiratory tract. These specimens can be taken using transtracheal aspiration, fibreoptic bronchoscopy, transthoracic needle puncture, or open lung biopsy. Such invasive procedures may also be necessary in the immunocompetent host, if therapy for a community acquired pneumonia or nosocomial pneumonia have failed and less invasive procedures are not likely to identify the cause of the disease.

As experience during the past years has shown, taking microbiological samples by protected brush, bronchoalveolar lavage and/or transbronchial lung biopsy are methods which combine a low rate of side-effects and a sufficient diagnostic yield when used in this context [222–224]. Also bronchoalveolar lavage alone is a sensitive method to establish the diagnosis of infection of the lower respiratory tract caused by bacteria [222, 223], mycobacteria [225], viruses [226] and other opportunistic infections of the lung (*e.g. Pneumocystis carinii* pneumonia) [227, 228] (summary in table 1).

Indications for bronchoalveolar lavage in patients with pulmonary infections

Immunodeficient or immunocompromised patients. In the clinical setting of an immunocompromised host (*e.g.* patients receiving immunosuppressive agents) or immunodeficient host (*e.g.* neutropenic patients) having pulmonary infiltrates suggesting lower respiratory tract infection, we recommend use of bronchoalveolar lavage as a means of obtaining samples from the lower respiratory tract for microbiological work-up. If the platelet count is normal, no clotting abnormalities are present and the patient is not at risk for mechanical ventilation, a transbronchial lung biopsy may be performed at the same time. Although TLB is not recommended in patients with thrombopenia or clotting abnormalities, a normal BAL has been safely applied even in thrombocytopenic and granulocytopenic patients after intensive cytotoxic therapy in conjunction with bone marrow transplantation [229].

In patients with an advanced HIV infection and suspected *Pneumocystis carinii* pneumonia an induced sputum [221] should precede the bronchoalveolar lavage. If sputum is nondiagnostic, BAL should be performed as soon as possible. In the majority of patients with HIV infection and pulmonary infiltrates the diagnosis can be established by BAL without additional transbronchial lung biopsy. BAL is reported to have a diagnostic yield to identify PC-infection of over 90%, followed by TLB with 75% and brush biopsy of only 32% [211]. Thus, considering the potential bleeding risk of an HIV infected patient with diffuse pulmonary Kaposi sarcoma, transbronchial lung biopsy should only be performed,

Table 1. – Microbiological diagnosis from BAL

	Technique, stains	Value	References
P. carinii	Wright-Giemsa Diff Quick Gomori-Grocott	80–90% sens.	[1, 3, 227–2302]
Cytomegalovirus	Virus cell inclusions		[231]
Herpes simplex	Immunofluoresc., Immuno-chem. DNA probe analysis		[226, 232] [233]
Mycobacteria	Ziehl-Neelsen Auramin-Rhodamin	atyp, typ. Tbc	[1, 227, 227]
Fungi	Silverstain Monoclonal antibod.	*Candida*, *Aspergillus*, *Alternaria*	[1, 229, 231, 234–237]
Bacteria	Gram stain		[238, 239]
	Semiquant. counting of CFU	Colonization or infection	[1, 224, 222]
Legionella	Direct immunofluoresc.		[240]

if prior investigations including BAL were nondiagnostic.

Immunocompetent patients. Bronchoalveolar lavage has been successfully used in this clinical setting also, in particular in patients suggestive for nosocomial pneumonia by help of Gram stains and bacterial cultures; semiquantitative counting of bacteria helps to differentiate between colonization and infection [222, 224, 239, 239]. Legionella infections can be detected either by direct immunofluorescence technique [240] or by bacterial culture.

Technique of bronchoalveolar lavage

Bronchoalveolar lavage is performed during fibreoptic bronchoscopy as described previously [1]. Although some local anaesthesia may be necessary to perform this procedure, the anaesthetic should not be instilled directly into the segment to be lavaged, as it may inhibit bacterial growth in the culture. Bronchoalveolar lavage should be performed in a segment which has been shown to be infiltrated on chest radiograph or from which purulent secretion is discharged during bronchoscopy. In adult patients a volume of 50–100 ml of saline should be used in this clinical setting. For the interpretation of laboratory results from BAL it may be helpful to obtain specimens from the oral cavity and hypopharynx at the time of the BAL. Supplemental oxygen should be given during the entire procedure and for at least 1 h after the bronchoscopy.

As immunocompromised patients with a pneumonia are at risk to develop respiratory failure, prior to BAL an arterial blood gas analysis should confirm that the patient is not at risk to develop respiratory distress during or after bronchoalveolar lavage. If arterial oxygen tension (Pao_2) despite supplemental oxygen is <65 mmHg bronchoalveolar lavage should be performed with care, reducing the volume of saline to be instilled. As the Pao_2 may drop substantially after bronchoalveolar lavage, adequate preparations have to be taken so that the patient can be intubated and ventilated if necessary. During the procedure vital signs, oxygen saturation and cardiac rhythm should be monitored continuously.

Work-up of specimens obtained by bronchoalveolar lavage

Specimens obtained from immunocompromised or immunodeficient patients should be processed as soon as possible, thus avoiding further contamination or missing such agents as anaerobic bacteria. Bronchoalveolar lavage fluid should be worked up for bacterial, fungal, opportunistic and viral infections. In addition the specimens should be examined by a cytopathologist to exclude a malignancy. The techniques used for these purposes are described in the technical recommendations and guidelines for BAL. In summary BAL fluid should be stained and cultured quantitatively for bacteria [224] using appropriate media, stained and cultured for mycobacteria including mycobacteria other than *M. tuberculosis* (MOTT) and for fungi. A *Pneumocystis carinii* infection should be ruled out by appropriate stains (Wright-Giemsa, silver stain, toluidine blue or monoclonal antibodies). Viral infections should be excluded using antibodies, viral cultures and DNA/RNA-probe analysis. If necessary electron microscopy enables a rapid differentiation of virus in bronchoalveolar lavage fluid.

In patients with HIV infection and diffuse pulmonary infiltrates a cell differential on a bronchoalveolar lavage slide may help to establish the diagnosis of lymphocytic interstitial pneumonia. Results from staining with appropriate antibodies and the demonstration of HIV in material from BAL may indicate the presence of nonspecific interstitial pneumonitis [242]

Interpretation of laboratory results

Results from BAL of immunocompromised or immunodeficient patients should be evaluated with care, considering the underlying disease, the history, the immunological status and the clinical features. In particular, the presence of cytomegalovirus (CMV) as shown by cultures or DNA-probe does not always indicate a clinically relevant infection. In case of detection of fungi or bacteria the clinician has to decide whether there is an infection, which should be treated, or a mere colonization. Quantitative cultures [224] may help to distinguish these two conditions.

Conclusions

BAL is the method of choice in diagnosis of opportunistic infections (bacteria, viruses, fungi, protozoa) of the lower respiratory tract in particular in immunodeficient or immunocompromised patients. Highest diagnostic yield is reported in the diagnosis of *P. carinii* pneumonia (>90%), which in many cases obviates the need of a lung biopsy. BAL can even be performed in patients with underlying respiratory insufficiency or in thrombocytopenic patients provided appropriate safety measures and selection of patients are undertaken. In patients with bacterial infections BAL may contribute to discrimination between bacterial colonization or true parenchymatous infection.

Pulmonary malignancies

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The major diagnostic techniques to obtain material for the diagnosis of cancer were, and remain direct forceps biopsy of bronchoscopically visible tumours and transbronchial biopsy for peripheral lesions.

Nevertheless, BAL can obtain material which can permit the cytological diagnosis of cancer. The criteria for the cytological diagnosis of cancer in the lung are well established [243]. However, since BAL is often performed and interpreted by pulmonologists [190] who are not trained cytologists and because the stains most often used by pulmonologists do not always reveal cytological detail, it is likely that the power of BAL to aid in the diagnosis of lung cancer has been underappreciated.

Table 1. – Examples of BAL used in the diagnosis of cancer

Type of cancer	Reference
Primary lung	
Squamous	[229, 244–248]
Adenocarcinoma	[229, 244–247]
Large cell	[229, 244, 246, 247]
Small cell	[229, 244–246]
Bronchoalveolar	[249–251]
Metastatic	
Solid tumours	[229]
Breast	[252]
Lymphangitic spread	[253, 254]
Haematological malignancy	
Hodgkin's	[255–257]
Non Hodgkin's lymphoma	[251, 256, 258, 259]
Leukaemia	[74, 229, 256]
Waldenstrom's	[260]
Myeloma	[256]
Mycosis fungoides	[261]

The exact circumstances in which lavage will be most important and the diagnostic yield comparable with other techniques are, as yet, unanswered questions. In addition, it should be recognized that BAL performed for other reasons may reveal malignant cells in cases where cancer is not suspected.

A rapidly enlarging collection of case reports and small series suggest that BAL can be of use in the diagnosis of a number of malignancies in the lung (table 1). With regard to primary lung cancers, there are six series (including unpublished data contributed by the co-authors of this document) which address the issue of diagnostic yield of BAL (table 2). Overall, the diagnostic yield was about 50% in these six series ranging from 14–69%. While the numbers available are small, the data available suggest that the diagnostic yield of BAL might be higher for bronchoalveolar cell carcinoma than for other cell types of primary pulmonary malignancy (table 3).

A high yield should also be expected in lymphangitic spread of metastatic cancer. The best technique of lavage to use for the diagnosis of cancer is unknown (table 4). It would be ideal to compare lavage with transbronchial biopsy, for example, for peripheral lesions, diffuse lesions and large central bronchoscopically visible lesions. Studies designed to address these issues are currently underway. While it is not possible to draw any firm conclusions, lavage can be of use in some cases of isolated peripheral nodules. It was also felt that lavage was particularly useful in diffuse lesions, such as those found with bronchoalveolar cell carcinoma. Thus, while lavage can clearly provide diagnostic material in a variety of clinical settings, its yield in specific settings, remains to be determined.

Finally, a number of staining methods are available (table 4), but the best laboratory techniques to use for the diagnosis of malignancy in bronchoalveolar lavage fluids are undetermined.

Table 2. – Diagnostic yield of bronchoalveolar lavage in lung cancer

Contributor	[Ref.]	No. of cases	No. with both BAL and diagnosis of cancer	No. of cases positive by BAL	% of cases positive by BAL
STRIZ*		471	430	225	52
WORTH*		146	99	37	37
BAGLIN	[262]	46	21	13	62
PIROZYNSKI*		124	124	44	35
LINDER	[229]	421	35	24	69
SCHABERG	[247]	31	21	3	14
Total			730	346	
	For sites mean = 45 For cases mean = 47				

*: unpublished results

Table 3. – Diagnostic yield for BAL in lung cancer

Cell type		% Yield
Bronchoalveolar cell carcinoma	11/12	92
Small cell	10/35	32
	2/3	
Squamous	0.9/49	27
	0.7/10	
Adenocarcinoma	11/20	66
	12/15	
Large cell	0/5	25
	3/7	

Data are reported for the various series available expressed as number of cases positive by BAL/number of cases of proven cancer undergoing BAL

Table 4. – Methods for the diagnosis of malignancy by bronchoalveolar lavage

Lavage technique: Lavage affected segment (CT may be helpful)		
Options*: "bronchial" and "alveolar" specimens for separate processing volume prior to/after brushings and biopsies		
Sample processing options*:		Smears Cytocentrifuge preparations Membrane filter preparations Cell pellets embedded in paraffin
Stains:	Routine*:	Papanicolaou Wright-Giemsa Haematoxylin and eosin
	Special:	Monoclonal antibodies for tumour markers

*: the "best" choice is undetermined; CT: computerized tomography.

A second limitation of lavage is that the cytological diagnosis of malignancy does not always correspond to the histologic pattern [253]. Thus, in the series of Linder, cytology agreed with biopsy in only 80% of cases. The major difficulty was in distinguishing large cell undifferentiated carcinoma from adenocarcinoma. A similar problem occurs with the severe dysplastic changes that can develop in airway epithelial cells in a variety of clinical circumstances including pneumonia, viral infections and following chemotherapy. These severe dysplastic changes can be very difficult to distinguish from malignant changes. These limitations of cytological methods must be considered when bronchoalveolar lavage is used in the diagnosis of lung cancer.

Several contributors to the current report have performed large series of bronchoalveolar lavage and have made a diagnosis of malignancy only very rarely. This has contributed to the impression that BAL has limited use in the diagnosis of cancer.

There are several reasons which may explain the low diagnostic yield at these centres: 1) case selection may have been very different at different centres; 2) pulmonologists interested in performing bronchoalveolar lavage for specific research goals may not have processed lavage specimens in a manner to maximize yield for malignancy. Some investigators, for example, throw away the first aliquot returned, which is relatively enriched for bronchial material. For malignancies originating in the bronchial tree, this may represent the material with the highest diagnostic yield. In addition, many investigators filter the fluid through loose-weave gauze in order to remove mucus. Malignant cells are often present as clumps and may be removed by such filtration procedures. Finally, many investigators have performed the procedure in patients with malignancy in order to investigate immunological abnormalities in these patients. They have intentionally lavaged sites not affected by the cancer. Thus, the relatively low diagnostic yield found by many investigators who have performed lavage for reasons other than to obtain diagnostic material, may reflect the interests of specific investigators rather than the utility of lavage to obtain material diagnostic of malignancy.

A number of tumour markers have been studied in bronchoalveolar lavage [246, 263]. While there is considerable interest among investigators in such markers, none has proved to be diagnostic. Thus, the use of these markers must be considered a research tool at present. Whether these markers will be helpful in following patients on a therapeutic protocol for malignancy is an interesting, but as yet unresolved, question. One investigator has suggested that cytological assessment of malignancy can be used for a similar purpose. Again, this must be considered a research undertaking. However, inasmuch as bronchoalveolar lavage might provide a means to assess efficacy of novel therapeutic strategies in lung cancer, it may become an important adjunct in clinical studies.

There is also a considerable interest in studying abnormalities in the patient with cancer. As such, a number of studies of bronchoalveolar lavage parameters have been undertaken in these patients. While these studies promise to provide some information as to why certain individuals develop malignancy and, perhaps, why these patients have increased incidences of lower respiratory tract infections, these studies are research studies.

It is difficult to summarize current consensus regarding the use of bronchoalveolar lavage for the diagnosis of lung cancer. Current practices vary from never performing this procedure for this indication to routinely performing this procedure for this indication. At institutions where this procedure is never performed, there is, obviously, no diagnostic yield associated with bronchoalveolar lavage. Centres where bronchoalveolar lavage has been found to be useful in the diagnosis of lung cancer are those where the procedure can be performed readily, the samples can be processed easily and trained personnel are available for the routine analysis of the specimens. In such a favourable setting, it would seem reasonable to include bronchoalveolar lavage in the diagnostic routine used to evaluate patients for lung cancer. This is particularly so considering that the procedure has exceedingly low morbidity, and the increased cost over performing a bronchoscopy with other diagnostic procedures is relatively low.

Bronchial asthma

L.M. Fabbri, V. De Rose, Ph. Godard, G.A. Rossi

In the past few years fibreoptic bronchoscopy and bronchoalveolar lavage fluid analysis have been extended to subjects with asthma and they are increasingly used to study airway cell profile and fluid components, as well as to study the characteristics of the harvested cells *in vitro* [198, 264, 265]. Initial studies were performed in stable asthmatics who were free of bronchospasm, and suggested that broncho- alveolar lavage could be safely performed as a research tool in carefully selected, asymptomatic asthmatic subjects. The guidelines provided by two international committees set up to evaluate the use of bronchoalveolar lavage and record its application, recognized and established the safety of the procedure in those selected patients [1, 266]. Many studies have been carried out without major complications on stable asthmatics before and after bronchoprovocation with allergens or occupational agents, and an international workshop on the use of bronchoalveolar lavage in asthma established the criteria to perform this procedure safely during the course of asthmatic responses to asthmogenic stimuli, as a research tool [266].

The general conclusion from the literature is that the bronchoalveolar lavage technique is safe in asthma, and that as long as reasonable guidelines are chosen for the selection of patients, the mortality is zero and the morbidity is very low. However, special care should be exercised in asthmatic patients with marked bronchial responsiveness, and supplemental oxygen delivery and electrocardiographic (ECG) monitoring is strongly advised in patients with severe underlying diseases or in any critical conditions [1, 198, 266]. Additional criteria are provided to select patients to undergo bronchoalveolar lavage following aerosol or local bronchoprovocation [266].

Clinical application of bronchoalveolar lavage in asthma

The analysis of cells, mediators, proteins and enzymes obtained from the alveolar spaces and the *in vitro* study of cells recovered from the respiratory tract can help to elucidate pathogenic mechanisms in asthma. In stable, mild asthmatics no distinctive cellular profile is diagnostic although eosinophils, neutrophils, epithelial cells, metachromatic cells and lymphocytes may be increased [198, 264, 265]. There seems to be no difference between the cellular profile of atopic and non-atopic stable asthmatics.

The major limitation of the standard technique of BAL is its intrinsic low sensitivity due to the fact that large volumes of fluid are instilled both in the airways and in the alveoli. To obtain true bronchial lavage by using lower volumes of fluid, new techniques have been recently developed to lavage isolated airway segments employing either a double balloon bronchoscope or a double balloon tipped catheter inserted through a double lumen bronchoscope (see following sections). This technique is extremly promising both because it is specific for the airways and because it already allowed the consistent recovery of cells and mediators before and after bronchoprovocation from the airways of asthmatic subjects, and some of the results seem to be specific for asthmatic airways [267–269].

The lack of specifity of bronchoalveolar lavage cell profile in asthma would discourage any clinical application of this procedure, especially since the diagnosis and monitoring of the activity of the disease seem to be accomplished effectively by using objective functional parameters, such as the measurement of airway responsiveness to nonspecific stimuli and the assessment of the spontaneous diurnal variability of airflow obstruction. Few patients with current active asthma have been evaluated. In most of the studies carried out in asthmatics the level of airway hyperresponsiveness, when it was measured, varied from mild to moderate, the subjects were defined as asymptomatic, and there was no attempt to evaluate the activity of the disease by using more than one functional parameter (*i.e.* the spontaneous variability of the airflow obstruction in addition to the level of nonspecific airway responsiveness). Thus, the results of bronchoalveolar lavage analysis in those patients may not be relevant to asthma but just to well-controlled asthmatics.

In formulating a reasonable position about the clinical use of bronchoalveolar lavage and its analysis in asthmatic subjects, one must acknowledge that it is still an experimental procedure, that needs further assessment and it must continue to be included as part of clinical research protocols. It may be proved to be clinically helpful in the evaluation of pulmonary infiltrates in asthmatics [264].

Bronchoalveolar lavage has also been considered for the therapy of status asthmaticus or life-threatening asthma attacks [198, 270]. The technique used for therapeutic lavages was not in fact bronchoalveolar lavage but just segmental washings, both because the procedure was not standardized and because the fluid was not analysed. Because of the limited experience and the lack of carefully designed clinical trials, the therapeutic segmental washings in patients with asthma must still be considered experimental in nature and performed in selected patients by well-trained physicians with an extensive experience in this field (see chapter: Therapeutic applications of BAL). One further promising application of bronchoalveolar lavage in asthma may be the assessment of the cellular response to antiasthma therapy [271, 272].

Conclusions

In agreement with the recent state-of-the-art paper and review articles, we believe that there is no indication at present for the use of bronchoalveolar lavage in clinical practice for the diagnosis, staging, monitoring or therapy of bronchial asthma. The only indication that may prove to be clinically helpful is the presence of pulmonary infiltrates in asthmatics, particularly for the diagnosis of allergic bronchopulmonary aspergillosis.

Chronic bronchitis and emphysema

E. Pozzi, V. De Rose, S.I. Rennard, L.M. Fabbri

Despite the widespread use of bronchoalveolar lavage (BAL) in several lung diseases, only a few studies have evaluated its usefulness in patients with chronic bronchitis and/or emphysema.

Bronchoalveolar lavage in the diagnosis of chronic bronchitis and emphysema

Chronic bronchitis is defined by the presence of symptoms, and emphysema by the presence of pathological enlargements of airspace with destructive changes in the walls, thus bronchoalveolar lavage has no application in the definition of the diagnosis of either disease. In addition, there is no indication at present for the use of such a procedure in clinical work for staging or monitoring the course of chronic bronchitis and emphysema because of the lack of specificity of the findings from bronchoalveolar lavage fluid analysis. Due to various degrees of severity of obstruction great care should be taken when lavaging these patients (see chapter on Side-effects and Safety of BAL).

Findings in bronchoalveolar lavage fluid from chronic bronchitis and emphysema

Asthma, chronic bronchitis and emphysema are grouped under the terminology of chronic obstructive pulmonary disease (COPD). At present, very little is known about the biochemical and cellular changes that occur in BAL in each stage of chronic bronchitis and emphysema, and it can be summarized in the following points: with the exception of smokers who have been well characterized and who are likely to have small airways disease, there is little information about bronchoalveolar lavage findings in subjects with obstruction of the small airways (small airways disease) and in subjects with simple chronic bronchitis.

In patients with COPD the recovered fluid is reduced to 10–40% of that instilled [273–276] and the bronchoalveolar lavage fluid contains an increased number of neutrophils as well as bronchial lavage fluid [274–277]. Bronchoalveolar neutrophilia is not specific for COPD, since it is present in smokers without COPD, patients with cystic fibrosis, and in some interstitial lung diseases [101, 198]. In BAL from patients with emphysema and alpha$_1$-PI, deficiency there is a severe neutrophilia (77.8%±18.4 of the differential count), suggesting high elastase burden in the alveolar lining fluid and reduced concentrations of alpha$_1$-PI, whereas the concentration of alpha$_2$-macroglobulin and antileucoproteases is normal [275].

Use of bronchoalveolar lavage in the therapy of chronic bronchitis and emphysema

At present, bronchial lavage has a limited role in the therapy of chronic bronchitis and emphysema. It may be used in some selected cases for removal of abundant secretions.

In the future it could provide a useful method of assessment of the effect of therapy. For example, according to the hypothesis that lung destruction in COPD is primarily mediated by a protease/antiprotease imbalance in the lower respiratory tract, the prevention of structural changes leading to severe functional impairment might be obtained by enhancing the antiprotease screen of the respiratory tract. Several pharmacological approaches have been investigated: 1) genetically engineered mutants of alpha$_1$-AT and low-molecular weight elastase inhibitors; and 2) alpha$_1$-AT that may be administrered in sufficient quantities by infusion to replete deficient patients. BAL might be used to evaluate the efficacy of this therapy, to verify whether adequate enzyme concentrations are reached in alveolar lining fluid [278, 279].

Conclusions

In conclusion, in agreement with recent review articles [101, 198], we believe that there is no indication at present for the use of bronchoalveolar lavage for the diagnosis, staging or monitoring of chronic bronchitis and emphysema because of the lack of specificity of the findings from bronchoalveolar lavage fluid analysis. However, bronchoalveolar lavage from patients with mild or moderate airflow obstruction can be safely accomplished for the investigation of the mechanisms involved in the development of the disease.

Therapeutic applications of BAL

C. Danel, D. Israel-Biet, U. Costabel, L.M. Fabbri, H. Klech

Although BAL had been used for therapeutic purposes prior to its use as a diagnostic procedure and the value of BAL in the exploration and management of some interstitial lung diseases is now well established, its place in therapy is controversially reported. As early as 1963, RAMIREZ *et al.* [280] were the first to perform a whole lung lavage (WLL) using a large volume of fluid in patients with pulmonary alveolar proteinosis. Since then, this technique has been proposed to remove any alveolar filling material in conditions such as alveolar proteinosis [196, 281], alveolar microlithiasis [282], acute silicosis [283], or accidental inhalation of radioactive particles [289, 205]. Its use has also been proposed in obstructive lung diseases [286] to remove the mucus secretions accumulated in the bronchial tree as in asthma [287, 288] or in cystic fibrosis [289, 290]. This lavage differs from the segmental BAL currently used for diagnostic or research purposes in that it is performed under general anaesthesia, and uses a much larger fluid volume. The actual procedure varies slightly from one centre to another and has not yet been standardized [196, 291]. WLL is a safe procedure as shown by the absence of chronic side-effects over periods as long as 25 yrs in patients treated for pulmonary alveolar proteinosis (PAP) [192]. On the other hand, its efficacy is known to be dependent on the type of disorder in which it is performed.

We will briefly review the main pathological conditions in which WLL is currently performed.

BAL in alveolar proteinosis

The benefit of therapeutic WLL is now well demonstrated in this disease. First proposed by RAMIREZ *et al.* [280], the technique has been slightly modified over the years. When the diagnosis of primary PAP is established, the decision to perform a therapeutic bronchopulmonary lavage should be based upon the patient's exercise tolerance and on his symptomatology, because spontaneous remission is always possible. When indicated, the performance of a WLL requires an experienced staff and considerable back-up facilities [196]. The first fluid samples to be recovered have a milky aspect which clears up progressively during the lavage. This treatment always improves the patient's symptoms [196, 281]. Some authors have shown a significant improvement of alveolar macrophage (AM) function after therapeutic WLL, demonstrating that the effect in AM function in PAP is reversible. Furthermore, this treatment could also reduce the rate of secondary infections [196, 281]. Although idiopathic forms of PAP are always improved by WLL, the periodicity of the need for therapeutic BAL varies widely from one patient to another, depending on the individual course of the disease.

In case the clinical symptoms do not dramatically improve after a whole lung lavage, a clinical and pathological search should be made for an associated condition; an open lung biopsy is then required to eliminate, for instance, acute silicosis, infections and/or malignancy [283, 293].

BAL in asthma

Mucus plugs are known to contribute to the severe hypoxaemia in patients with status asthmaticus due to large ventilatory defect. These plugs can be removed by suction through a bronchoscope after the instillation of saline or acetylcysteine [287, 288]. However, this procedure was thought to have a high risk/benefit ratio. Some investigators have markedly improved the benefit of this technique by limiting the indications and through technical modifications. Clinical benefit is likely if tenacious mucus plugging or tracheobronchial casts are present. Nevertheless, despite this study [288], it seems that WLL in patients with severe asthma must still be considered as experimental in nature and performed in selected patients, by well-trained physicians with an extensive experience in this field and only in the context of an intensive care unit.

BAL in pneumoconiosis

It is well known that inhaled inorganic dust damages the lung by inducing an inflammatory reaction that progressively leads to fibrosis. WLL has been proposed in order to remove the irritating dust before this irreversible damage occurs especially in the acute form of silicosis [294]. The lavage fluid is usually striking with its black or brown colour and numerous alveolar macrophages containing dust particles. It seems that the procedure results in rapid symptomatic improvement but without modification of the pulmonary function or the prognosis [294].

BAL in inhalation of radioactive particles

The benefit of WLL in human contamination is not yet clearly defined [284, 285]. Experimental studies on dogs and baboons have been carried out over the last twenty years to determine the efficacy of WLL in the removal of such particles. It seems that, although the longer the radioactive material is present in the lung, the greater the dose delivered, WLL should not be performed in the early stages of contamination since it can prevent the usual physiological clearance of inhaled particles from the upper respiratory tract. WLL seems to be indicated in levels of contamination inducing acute effects, while its value in patients with lesser exposure is not clearly established.

Other therapeutic applications of BAL

WLL has been proposed in the treatment of some other pulmonary disorders such as alveolar microlithiasis or exogenous lipoidosis, with some clinical but without any objective functional or radiological improvement [282].

In cystic fibrosis (CF), the benefit of WLL is also difficult to evaluate. It was expected that periodical repeated WLL could, if not arrest, at least slow down the progressive deterioration of lung function caused by the accumulation of bronchial secretions [289, 290]. Some authors have proposed WLL using antifungal drugs as a local treatment of aspergillosis, a frequent complication of CF [290]. This requires further investigation.

Conclusions

The therapeutic value of BAL is now perfectly established in alveolar proteinosis, which remains the only definite indication of this procedure. In other lung disorders, this technique still has a risk/benefit ratio which does not argue for its use in routine clinical practice. Its indication should be discussed for each patient and performed by an experienced staff in the context of an intensive care unit.

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