CHAPTER 4

The role of bronchoalveolar lavage cellular analysis in the diagnosis of interstitial lung diseases

*P. Spagnolo**, L. Richeldi**, G. Raghu*[#]

**Centre for Rare Lung Diseases, University of Modena and Reggio Emilia, Modena, Italy.* [#]*Division of Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA, USA.*

Correspondence: G. Raghu, Division of Pulmonary and Critical Care Medicine, Campus Box 356522, UW Medical Center, University of Washington, Seattle, WA 98195-6522, USA. E-mail: graghu@u.washington.edu

Interstitial lung diseases (ILDs) represent a very broad and heterogeneous group of acute and chronic lung disorders with variable degrees of inflammation and fibrosis, that predominantly affect the distal pulmonary parenchyma including the confined interstitial space bounded by the epithelial and endothelial basement membranes of the alveolar wall. Based on the similarities of several clinical features, they are lumped together as ILDs. However, there are significant differences in aetiology, in abnormal features of the lung on high-resolution computed tomography (HRCT) of the chest and under microscopy, and in the clinical course, prognosis, treatment and response to treatment of specific subgroups. Idiopathic interstitial pneumonias (IIPs) are a specific subgroup of ILDs of unknown aetiology, with distinct histological features [1]. The diagnostic algorithm for ILDs and IIPs requires a very thorough evaluation including a full medical history (with occupational and environmental exposures or drug reactions), family medical history, physical examination, laboratory investigations (including autoantibodies), lung function tests and imaging studies (chest radiography and HRCT). If these initial diagnostic interventions fail to produce a specific diagnosis for the ILD, additional tests that are considered ''invasive'', such as bronchoscopy with bronchoalveolar lavage (BAL) cellular analyses, transbronchial lung biopsy and surgical lung biopsy are helpful. In this context, bronchoscopy is minimally invasive and generally well tolerated with very low overall morbidity and mortality [2]. BAL is conventionally performed in a bronchopulmonary segment of one lobe only, either the right middle lobe or one of the basal segments of the right lower lobe. While a single-site BAL cellular profile is assumed to be representative of the lung as a whole in ILD (because interstitial pneumonias by definition involve all bronchopulmonary segments [3]), some evidence suggests that this diagnostic procedure might be more useful if it was targeted to one of the pulmonary segments most affected, as identified by chest HRCT [4, 5]. This is particularly true in fibrotic diseases, in which areas of extensive honeycomb lung yield a small recovery of BAL fluid, representing the cellular profiles of end-stage fibrotic lung: such data may not yield useful clues for the diagnosis of ILD. Routine processing of BAL fluid cellular analyses for patients with ILD includes total and differential cell counts and the determination of lymphocyte subsets as well as the morphological appearances of cells, besides cultures and special stains for infection in the appropriate clinical setting. Despite the wide and routine use of BAL during bronchoscopy as a diagnostic intervention for evaluation of infection in the distal

Eur Respir Mon, 2009, 46, 36–46. European Respiratory Monograph; ISSN 1025-448x.

airways and pulmonary parenchyma, there is significant variability in the technical aspects of the BAL procedure. The volume of saline instilled to retrieve cells from the pulmonary parenchyma, the positioning of the patient, the suction applied and the processing of the BAL fluid for cellular analyses have not been standardised globally, something that may contribute to the varying results from different laboratories and centres. Other confounding factors, such as smoking history, age, and medications that might influence the trafficking of the inflammatory cells into the lung (*e.g.* corticosteroids and other immunomodulating agents) need to be taken into consideration when interpreting the cellular profile observed in the BAL. An international consensus for guidelines regarding BAL procedure, technique, processing and the interpretation of the inflammatory cellular patterns observed in BAL from patients with ILD is needed. Such a consensus might iron out current differences of clinical practice in the utilisation of BAL for cellular analyses in the evaluation and management of ILDs.

Inflammatory cellular patterns in BAL

In the appropriate clinical setting, BAL findings may be diagnostic *per se* [6]. BAL cellular profiles may help to narrow the differential diagnoses of ILDs and to direct specific diagnostic interventions (table 1).

BAL samples obtained from healthy nonsmoker subjects contain 80–90% alveolar macrophages (AMs), 5–15% lymphocytes, 1–3% polymorphonuclear neutrophils (PMNs), <1% eosinophils, and <1% mast cells [7]. In ILD, the differential cell counts and specific BAL lavage features can be variable, nonspecific and insensitive. Dust particles and birefringent material within the AMs or elevated asbestos body counts suggest occupational exposures (fig. 1), while foamy or ''lipid-laden'' macrophages suggest the diagnosis of hypersensitivity pneumonitis (HP), drug-induced pneumonia or lipoid pneumonia [7]. In alveolar proteinosis, the combination of grossly milky/turbid BAL fluid, periodic acid–Schiff (PAS)-positive acellular oval bodies and foamy

Table 1. – Bronchoalveolar lavage (BAL) features diagnostic of specific interstitial lung diseases

BAL features	Diagnosis
Gross appearance	
Milky fluid, periodic acid–Schiff-positive acellular corpuscles, foamy macrophages	Alveolar proteinosis
Grossly bloody. Increased intensity with serial aliquots	Alveolar haemorrhage
Oily material that layers out above aqueous phase	Lipoid pneumonia
Cloudy material that clears with low-speed centrifugation	Alveolar microlithiasis
Microscopic features	
Specific pathogen by stains or cultures	Infection
Free red blood cells, haemosiderin-laden macrophages, fragmented red blood cells in the alveolar macrophages	Alveolar haemorrhage
Lymphocytosis (≥25%)	Hypersensitivity pneumonitis, sarcoidosis, chronic beryllium disease
CD4:CD8 lymphocyte ratio >3.5 (in the absence of mixed cellular patterns)	Sarcoidosis
Eosinophils >25%	Eosinophilic pneumonia
Dust particles and birefringent material in the alveolar macrophages, elevated asbestos body count	Occupational exposure
Malignant cells	Malignancy
Positive lymphocyte proliferation test to beryllium	Beryllium exposure
CD1a-positive Langherans' cells >5%	Pulmonary Langherans' cell histiocytosis

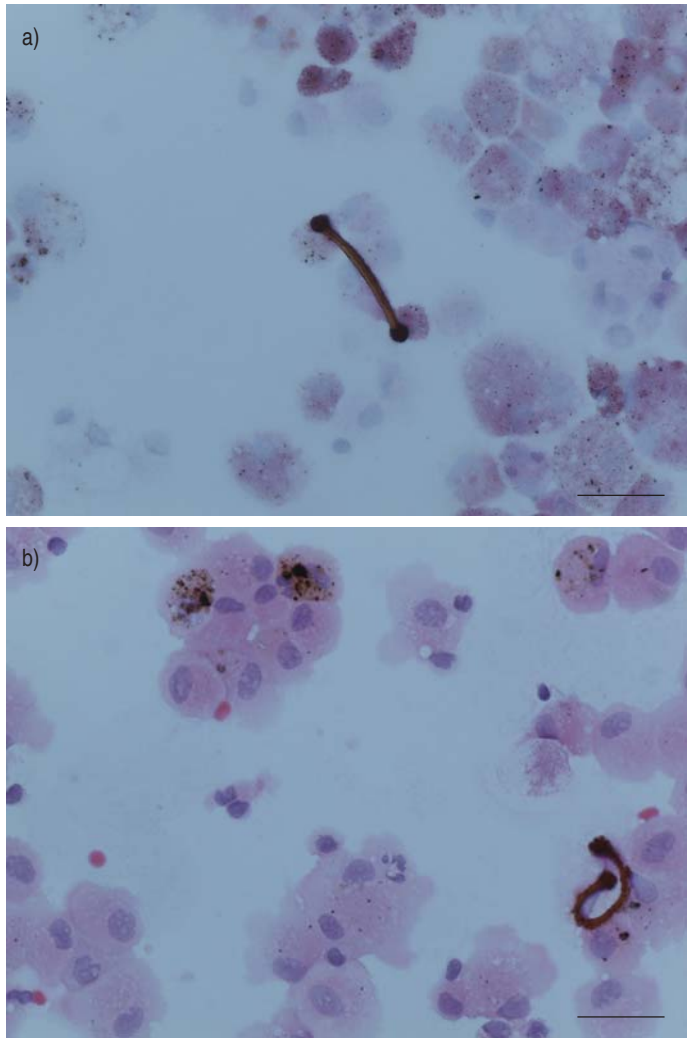

Fig. 1. – Typical a) asbestos and b) ferruginous bodies in a bronchoalveolar lavage specimen obtained from a patient with significant asbestos exposure (haematoxylin and eosin stain). Scale bars=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

macrophages under light microscopy is virtually pathognomonic of the disease, and obviates the need for surgical lung biopsy (fig. 2) [8].

Lymphocytic cellular pattern in BAL

A lymphocytic BAL pattern is commonly seen in granulomatous lung diseases, such as sarcoidosis and HP, and in the context of immune reactions of the lung to some drugs (fig. 3) [9]. An increased lymphocyte count – predominantly driven by activated T-helper cells – with a CD4:CD8 ratio >3.5 is regarded as typical of pulmonary sarcoidosis, and is considered generally sufficient to secure the diagnosis of sarcoidosis in the appropriate clinical setting (*e.g.* bilateral hilar adenopathy, uveitis or erythema

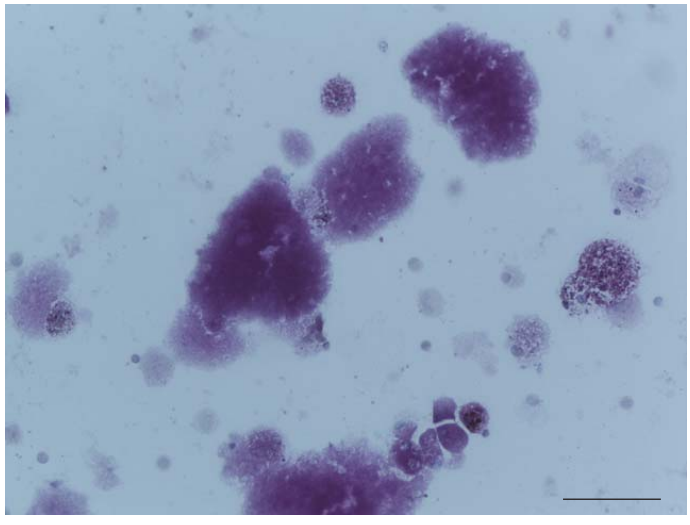

Fig. 2. – Amorphous granular material and engulfed macrophages in pulmonary alveolar proteinosis (periodic acid–Schiff stain). Scale bar=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

nodosum). However, both the number of lymphocytes in BAL and the BAL CD4:CD8 ratio can be variable [10–12]. The majority of sarcoidosis cases display an isolated BAL lymphocytosis, while a raised neutrophil count appears to correlate with more severe disease (as assessed by chest radiograph) and need for therapy [13, 14]. Conversely, in HP not only the number of lymphocytes but also the absolute neutrophil and eosinophil counts may be significantly increased [15]. BAL lymphocytosis >50% generally raises a suspicion for the diagnosis of HP and patients with HP may exhibit either decreased or increased CD4:CD8 ratio [16]. Methotrexate pneumonitis and chronic beryllium disease may also display an increased number of CD4-positive BAL lymphocytes [17, 18]. Unfortunately, a lymphocytic BAL does not distinguish between farmers with HP and exposed asymptomatic farmers who may remain asymptomatic and not progress to clinically relevant disease, despite their elevated BAL lymphocyte count [19]. Similarly, patients treated with amiodarone and without clinically significant pulmonary involvement may have BAL findings suggestive of a subclinical alveolitis [20]. BAL lymphocytosis appears also to be common in the cellular variant of nonspecific interstitial pneumonia (NSIP) [21]. Finally, a subclinical lymphocytic cellular pattern in BAL has been reported in Wegener's granulomatosis, Crohn's disease, and primary biliary cirrhosis [22, 23].

Neutrophilic cellular pattern in BAL

Elevated BAL PMN counts are commonly found in idiopathic pulmonary fibrosis (IPF), asbestosis, acute respiratory distress syndrome (ARDS), aspiration pneumonia, subacute HP and bronchiolitis obliterans (BOOP), as well as in pulmonary infections (fig. 4) [24, 25]. Although nonspecific, an increased neutrophil count in the appropriate clinical setting (with or without a mild eosinophilia) is observed in the BAL of 70–90% of patients with IPF [26]. Increased BAL neutrophils have been related to the extent of disease on HRCT and the severity of functional impairment [27].

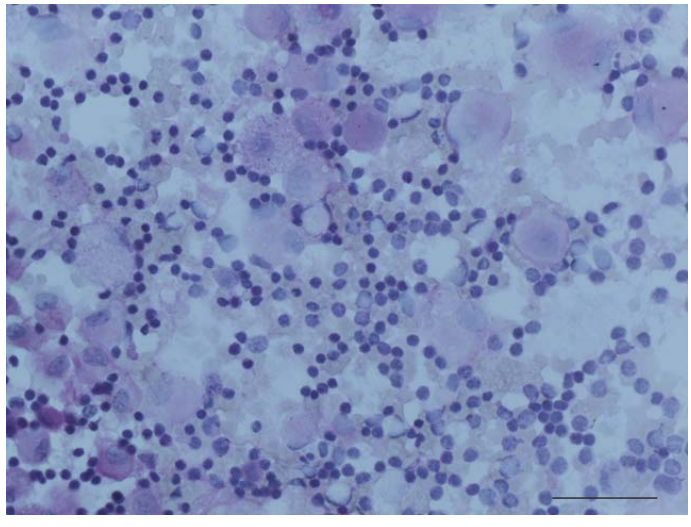

Fig. 3. – Bronchoalveolar lavage fluid with lymphocyte predominance in hypersensitivity pneumonitis (periodic acid–Schiff stain). Scale bar=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

Eosinophilic cellular pattern in BAL

In the absence of asthma and parasitic infestations, a predominantly eosinophilic BAL cellular pattern is highly suggestive of eosinophilic pneumonia, Churg–Strauss syndrome, allergic bronchopulmonary aspergillosis or drug-induced lung reaction (fig. 5) [28]. While BAL eosinophilia and increased AMs are common in patients with pulmonary Langherans' cell granulomatosis, the diagnosis can be secured by the presence of >5% Langherans' cells in BAL, identified by monoclonal antibodies

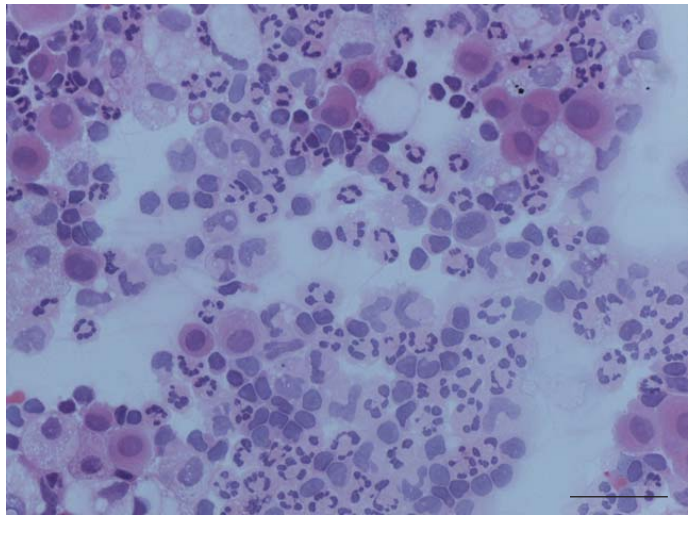

Fig. 4. – Bronchoalveolar lavage specimen showing increased number of total cells, predominantly neutrophils (haematoxylin and eosin stain). Scale bar=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

directed against the CD1a antigen [29, 30]. An increased BAL eosinophil count can also be observed in IPF, HP and collagen vascular disease-associated pulmonary fibrosis [28]. Despite the nonspecificity of eosinophilic cellular pattern in BAL, eosinophilia >25% raises the suspicion of ILD primarily mediated by eosinophils (*i.e.* eosinophilic pneumonia) [31].

Other inflammatory cellular patterns in BAL

Plasma cells are absent from normal BAL; as such, their presence accompanied by "foamy macrophages" and raised lymphocyte count is highly suggestive of either HP (especially the acute form) or drug toxicity [32]. Other ILDs associated with the presence of plasma cells in BAL include cryptogenic organising pneumonia and chronic eosinophilic pneumonia [33].

Mast cells have been implicated in the pathogenesis of lung inflammation and fibrosis. An increased number of mast cells in BAL has been observed in IPF, HP, and, to a much lesser extent, in sarcoidosis, and seems to relate to advanced or progressive disease [34].

BAL analysis is helpful in diagnosing alveolar haemorrhage syndromes, including Goodpasture's syndrome, Wegener's granulomatosis, systemic lupus erythematosus and other vasculitides, idiopathic pulmonary haemosiderosis, pulmonary capillaritis and collagen vascular disease (fig. 6). In the absence of coagulopathy, the gross appearance of increasingly haemorrhagic returns of the BAL fluid in sequential aliquots and/or the presence of fragmented red blood cells or iron in the AMs raises the suspicion of alveolar haemorrhage.

In addition, if mixed cellular patterns are present, the predominant cellular pattern might offer a clue to the diagnosis, although in these circumstances, invasive procedures such as lung biopsy may be required to make a specific diagnosis.

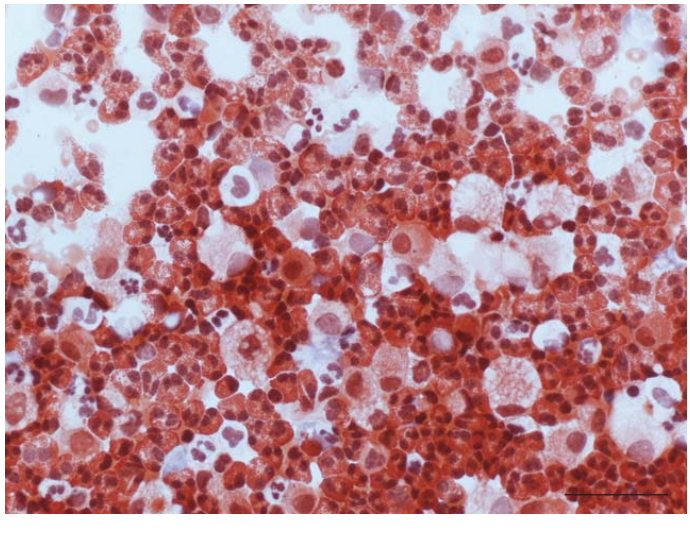

Fig. 5. – Predominantly eosinophilic bronchoalveolar lavage specimen in eosinophilic pneumonia (Pagoda red stain). Scale bar=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

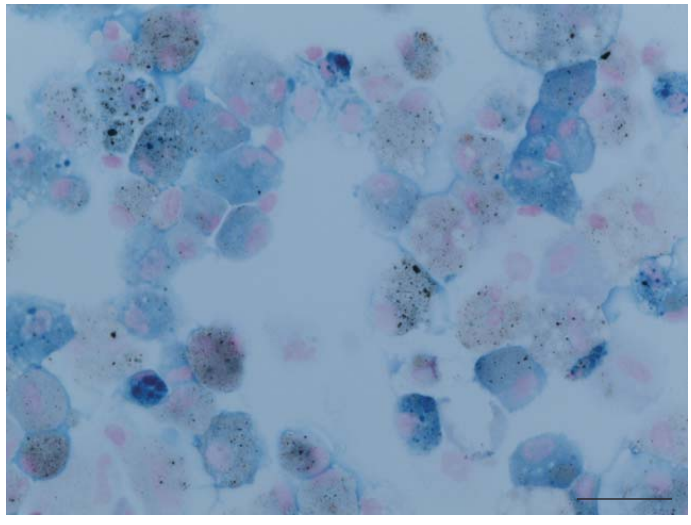

Fig. 6. – Bronchoalveolar lavage specimen showing iron-engulfed pigmented macrophages in alveolar haemorrhage (Perls stain). Scale bar=100 μm. Courtesy of G. Rossi, University of Modena and Reggio Emilia, Modena, Italy.

Usefulness of BAL cellular patterns in diagnosing IPF

IPF is a progressive and often fatal fibro-proliferative lung disorder of unknown aetiology characterised by the histopathological and HRCT pattern of usual interstitial pneumonia (UIP). The existing American Thoracic Society (ATS)/European Respiratory Society (ERS) consensus statement suggests major criteria (exclusion of known causes of interstitial lung disease; abnormal pulmonary function with restriction and/or decreased gas transfer; bibasal reticular abnormalities on HRCT scans or chest radiograph; BAL or transbronchial lung biopsy not suggesting any other disease), and minor criteria (age >50 yrs; insidious onset of otherwise unexplained dyspnoea; duration of illness >3 months; bibasal inspiratory crackles on auscultation) for the clinical diagnosis of IPF. It is likely that these criteria will be simplified in the updated version of the ATS/ERS IPF consensus statement, which is yet to be published. If patients do not meet these major and minor criteria, a surgical lung biopsy is necessary for a confident diagnosis [1]. However, with increasing recognition of the classical features in HRCT images of the lung that are associated with a UIP histopathological pattern, the diagnosis of IPF in the appropriate clinical setting may be accomplished without the need for lung biopsy [35–37]. It is anticipated that the awaited new evidence-based guidelines for managing IPF will clarify the diagnostic criteria for IPF based on UIP patterns recognised in chest HRCT scans.

One of the problems with IPF is that the disease has no pathognomonic clinical, biochemical, BAL cellular or pathological features [38–40]. An increase in the BAL lymphocyte count, which has been associated with a positive clinical response to steroid treatment and better outcome, is found in 10–20% of patients with IPF [41, 42]. However, because these latter data come from historical studies, some of these patients most probably had NSIP, a more benign form of interstitial pneumonia, which more recently has been recognised as a separate entity [43]. Conversely, a lone BAL lymphocytosis is uncommon in IPF and, when present, should raise the suspicion of alternative diagnoses, such as granulomatous infectious diseases, sarcoidosis, HP, BOOP, NSIP or lymphocytic interstitial pneumonia (LIP).

BAL findings, although nonspecific to IPF, are quite distinct from differential cell counts in sarcoidosis or HP. Conversely, distinguishing IPF from the fibrotic variant of NSIP solely based on the BAL cellular profile is far more difficult. Recently, RYU *et al.* [44] retrospectively evaluated the role of BAL in separating NSIP from UIP in a large patient population and observed that UIP was characterised by a higher neutrophil count (7%) and lower lymphocyte count (5%) than NSIP (3% and 29%, respectively). However, these results are in contrast with a previous study by VEERARAGHAVAN *et al.* [45], who reported that BAL had neither a diagnostic role nor prognostic value in a smaller cohort of patients with either IPF or idiopathic NSIP [45]. More recently, OHSHIMO *et al.* [46] observed that in six out of 74 patients suspected to have IPF based on HRCT, pulmonary function and clinical findings, the presence of a predominantly lymphocytic BAL raised the suspicion of an alternative diagnosis. In this study, IPF patients displayed a BAL lymphocyte count <30%, while five patients (6%) did not have BAL neutrophilia. Subsequently, a retrospective analysis of a dataset of IPF patients (n=42) from another centre [47] showed that none of them had >30% lymphocytosis, with three patients (7%) displaying only mild lymphocytosis (range 14–17%), far below the 30% cut-off adopted by OHSHIMO *et al.* [46] and consistent with previous data showing that an increase in BAL lymphocytes alone may be present in up to 10% of IPF patients [48]. However, it is unclear whether BAL adds significant diagnostic specificity to a thorough and very careful exposure history and other clinical and laboratory findings (*e.g.* serum precipitins), thus highlighting the importance of excluding known causes of interstitial pneumonia at the beginning of the diagnostic process [46]. The diagnostic gold standard of IPF is therefore an integrated clinical–radiological–pathological evaluation.

Conclusions

BAL is a well-tolerated procedure and has gained an important role in the diagnostic evaluation of new-onset ILDs. The decision to perform bronchoscopy and BAL should be guided by clinical presentation and the patient's functional status as well as local technical expertise, both in performing the procedure and in interpreting the BAL cellular component. In fact, one of the most commonly encountered problems with interpreting BAL results has been variability between centres, which highlights the importance of following standard protocols. While there is a need for a consensus statement among international experts to provide guidelines for the utility of BAL cellular analyses in clinical practice worldwide, a number of specific conditions, such as infections, malignancy and some rare ILDs, can be diagnosed by BAL analyses, thus avoiding more invasive procedures. In addition, even when nondiagnostic, BAL cellular analysis usually adds valuable information to the clinical and radiological data and helps to narrow the differential diagnosis. On the other hand, the diagnostic usefulness of BAL in IPF is limited, and the most important application of BAL in the evaluation of patients with suspected IPF appears to be the exclusion of chronic HP.

BAL is an invaluable research tool for ILD, providing information regarding immune effector cells that accumulate in the alveoli and their noncellular products. It is hoped that with a new consensus among international experts, the procedure for cellular analyses and interpretation will be standardised and thus the clinical utility of BAL cellular analyses will be widely accepted worldwide. With the increasing availability of proteomics, the importance of biomarkers of ILD will hopefully become clear in the near future. From a clinical standpoint, it is essential that BAL findings be interpreted by experts in the context of clinical and radiological data.

Summary

Fibreoptic bronchoscopy with bronchoalveolar lavage (BAL) is a minimally invasive and generally well tolerated procedure with very low morbidity and mortality. Importantly, the cells within the distal airways and alveolar surface provide potentially useful information regarding the pulmonary microenvironment involved with a disease process or in close proximity to it. This approach will continue to be helpful as more details of the normal lung are found and the pathogenesis of pulmonary diseases is explored.

BAL has gained an important role in the evaluation of interstitial lung disease (ILD), which often represents a diagnostic dilemma. In a number of conditions, such as infections, malignancy and some rare ILDs, positive findings by BAL analysis – when present – may be sufficient to formulate a specific diagnosis, thus avoiding the need for more invasive procedures. In others, BAL may substantiate a variety of alternative specific diagnoses, provided appropriate laboratory studies are performed to identify the key features. Therefore, even when nondiagnostic, BAL cellular analysis usually provides valuable information in addition to the clinical and radiological data, and helps to narrow the differential diagnosis based on cellular patterns. Conversely, the diagnostic usefulness of BAL in idiopathic pulmonary fibrosis (IPF) – the most aggressive idiopathic interstitial pneumonia – is limited, and its most important application in the evaluation of patients with suspected IPF appears to be the exclusion of alternative diagnoses.

While uniform and standardised techniques for collecting and analysing BAL fluid are needed, it is imperative that BAL cellular findings always be interpreted in the context of clinical and radiological data.

Keywords: Bronchoalveolar lavage, bronchoscopy, diagnosis, interstitial pneumonia, monitoring.

Statement of interest
None declared.

References

1. American Thoracic Society/European Respiratory Society. ATS/ERS international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2002; 165: 277–304.
2. Credle WF Jr, Smiddy JF, Elliott RC. Complications of fiberoptic bronchoscopy. *Am Rev Respir Dis* 1974; 109: 67–72.
3. Peterson MW, Nugent KM, Jolles H, *et al.* Uniformity of bronchoalveolar lavage in patients with pulmonary sarcoidosis. *Am Rev Respir Dis* 1988; 137: 79–84.
4. Agusti C, Xaubet A, Luburich P, *et al.* Computed tomography-guided bronchoalveolar lavage in idiopathic pulmonary fibrosis. *Thorax* 1996; 51: 841–845.
5. Baughman RP, Raghu G. Bronchoalveolar cellular analysis in scleroderma lung disease: does Sutton's law hold? *Am J Respir Med* 2008; 177: 2–3.
6. Reynolds HY. Use of bronchoalveolar lavage in humans: past necessity and future imperative. *Lung* 2000; 178: 271–293.

7. Klech H, Hutter C. Clinical guidelines and indications for bronchoalveolar lavage (BAL): report of the European Society of Pneumology Task Group on BAL. *Eur Respir J* 1990; 3: 937–974.
8. Costabel U, Guzman J. Alveolar proteinosis. *In*: Olivieri D, du Bois RM, eds. Interstitial Lung Diseases. *Eur Respir Mon* 2000; 14: 194–205.
9. Daniele RP, Elias JA, Epstein PE, *et al.* Bronchoalveolar lavage: role in the pathogenesis, diagnosis, and management of interstitial lung disease. *Ann Intern Med* 1985; 102: 93–108.
10. Costabel U. CD4:CD8 ratios in bronchoalveolar lavage fluid: of value for diagnosing sarcoidosis? *Eur Respir J* 1997; 10: 2699–2700.
11. Kantrow S, Meyer KC, Kidd P, *et al.* The CD4/CD8 ratio in BAL fluid is highly variable in sarcoidosis. *Eur Respir J* 1997; 10: 2716–2721.
12. Winterbauer RH, Lammert J, Selland M, *et al.* Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. *Chest* 1993; 104: 352–361.
13. Drent M, Jacobs JA, De Vries J, *et al.* Does the cellular bronchoalveolar lavage fluid profile reflect the severity of sarcoidosis? *Eur Respir J* 1999; 13: 1338–1344.
14. Ziegenhagen MW, Rothe ME, Schlaak M, *et al.* Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. *Eur Respir J* 2003; 21: 407–413.
15. Drent M, van Nierop MA, Gerritsen FA, *et al.* A computer program using BALF-analysis results as a diagnostic tool in interstitial lung diseases. *Am J Respir Crit Care Med* 1996; 153: 736–741.
16. Costabel U, Guzman J. Bronchoalveolar lavage in interstitial lung disease. *Curr Opin Pulm Med* 2001; 7: 255–261.
17. Schnabel A, Richter C, Bauerfeind S, *et al.* Bronchoalveolar lavage cell profile in methotrexate induced pneumonitis. *Thorax* 1997; 52: 377–379.
18. Rose CS, Newman LS. Hypersensitivity pneumonitis and chronic beryllium disease. *In*: Schwartz MI, King TE, eds. Interstitial Lung Disease. St Louis, Mosby-Year Book, 1993; pp. 231–253.
19. Laviolette M, Cormier Y, Loiseau A, *et al.* Bronchoalveolar mast cells in normal farmers and subjects with farmer's lung: diagnostic, prognostic, and physiologic significance. *Am Rev Respir Dis* 1991; 144: 855–860.
20. Camus P, Foucher P, Bonniaud P, *et al.* Drug-induced infiltrative lung disease. *Eur Respir J* 2001; 18: Suppl. 32, 93s–100s.
21. Nagai S, Kitaichi M, Itoh H, *et al.* Idiopathic non-specific interstitial pneumonia/fibrosis: comparison with idiopathic pulmonary fibrosis and BOOP. *Eur Respir J* 1998; 12: 1010–1019.
22. Schnabel A, Reuter M, Gloeckner, *et al.* Bronchoalveolar lavage cell profiles in Wegener's granulomatosis. *Respir Med* 1999; 93: 498–506.
23. Camus P, Colby TV. The lung in inflammatory bowel disease. *Eur Respir J* 2000; 15: 5–10.
24. Meyer KC. The role of bronchoalveolar lavage in interstitial lung disease. *Clin Chest Med* 2004; 25: 637–649.
25. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. *Am Rev Respir Dis* 1990; 141: S169–S202.
26. Bradley B, Branley HM, Egan JJ, *et al.* Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. *Thorax* 2008; 63: Suppl. 5, v1–v58.
27. Wells AU, Hansell DM, Rubens MB, *et al.* Fibrosing alveolitis in systemic sclerosis. Bronchoalveolar lavage findings in relation to computed tomographic appearance. *Am J Respir Crit Care Med* 1994; 150: 462–468.
28. Allen JN, Davis WB. Eosinophilic lung diseases. *Am J Respir Crit Care Med* 1994; 150: 1423–1438.
29. Vassallo R, Ryu JH, Colby TV, *et al.* Pulmonary Langherans'-cell histiocytosis. *N Engl J Med* 2000; 342: 1969–1978.
30. Tazi A. Adult pulmonary Langerhans' cell histiocytosis. *Eur Respir J* 2006; 27: 1272–1285.
31. Allen JN, Pacht ER, Gadek JE, *et al.* Acute eosinophilic pneumonia as a reversible cause of noninfectious respiratory failure. *N Engl J Med.* 1989; 321: 569–574.

32. Drent M, van Velzen Blad H, Diamant M, *et al.* Differential diagnostic value of plasma cells in bronchoalveolar lavage fluid. *Chest* 1993; 103: 1720–1724.
33. Drent M, Baughman RP, Meyer KC. Bronchoalveolar lavage. *In:* Costabel U, du Bois RM, Egan JJ, eds: Diffuse Parenchymal Lung Disease. *Prog Respir Res* 2007; 36: 58–67.
34. Pesci A, Bertorelli G, Gabrielli M, *et al.* Mast cells in fibrotic lung disorders. *Chest* 1993; 103: 989–996.
35. Raghu G, Mageto YN, Lockhart D, *et al.* The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: a prospective study. *Chest* 1999; 116: 1168–1174.
36. Hunninghake GW, Zimmerman MB, Schwartz DA, *et al.* Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2001; 164: 193–196.
37. Flaherty KR, King TE Jr, Raghu G, *et al.* Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? *Am J Respir Crit Care Med* 2004; 170: 904–910.
38. Nicholson AG, Addis BJ, Bharucha H, *et al.* Inter-observer variation between pathologists in diffuse parenchymal lung disease. *Thorax* 2004; 59: 500–505.
39. Flaherty KR, Travis WD, Colby TV, *et al.* Histopathologic variability in usual and nonspecific interstitial pneumonias. *Am J Respir Crit Care Med* 2001; 164: 1722–1727.
40. Rudd RM, Haslam PL, Turner-Warwick M. Cryptogenic fibrosing alveolitis relationships of pulmonary physiology and bronchoalveolar lavage to treatment and prognosis. *Am Rev Respir Dis* 1981; 124: 1–8.
41. Watters LC, Schwarz MI, Cherniack RM, *et al.* Idiopathic pulmonary fibrosis: pretreatment bronchoalveolar lavage cellular constituents and their relationships with lung histopathology and clinical response to therapy. *Am Rev Respir Dis* 1987; 135: 696–704.
42. Haslam PL, Turton CW, Lukoszek A, *et al.* Bronchoalveolar lavage fluid cell counts in cryptogenic fibrosing alveolitis and their relation to therapy. *Thorax* 1980; 35: 328–339.
43. Travis WD, Hunninghake G, King TE Jr, *et al.* Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. *Am J Respir Crit Care Med* 2008; 177: 1338–1347.
44. Ryu YJ, Chung MP, Han J, *et al.* Bronchoalveolar lavage in fibrotic idiopathic interstitial pneumonias. *Respir Med* 2007; 101: 655–660.
45. Veeraraghavan S, Latsi PI, Wells AU, *et al.* BAL findings in idiopathic nonspecific interstitial pneumonia and usual interstitial pneumonia. *Eur Respir J* 2003; 22: 239–244.
46. Ohshimo S, Bonella F, Cui A, *et al.* Significance of bronchoalveolar lavage for the diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2009; 179: 1043–1047.
47. Spagnolo P, Luppi F, Rossi G, *et al.* To BAL or not to BAL: is this a problem in diagnosing IPF? *Am J Respir Crit Care Med* 2009; 180: 379–380.
48. Schwartz DA, Helmers RA, Galvin JR, *et al.* Determinants of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1994; 149: 450–454.