Chapter 6

Bronchoalveolar lavage

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In this chapter we will discuss the role of BAL in IPF from clinical and research perspectives, presenting a review of the most recent advances in both areas. Although guidelines on the diagnosis and management of IPF do not recommend the use of BAL, its use is argued in excluding differential diagnosis and in patients without a confident diagnosis on HRCT, which is driven by the centre's experience and expertise. As a research tool, BAL has been useful in the study of the pathogenesis of pulmonary fibrosis, as well as in the identification of disease subtypes and response to therapy. After discussing the safety of the technique and less invasive alternatives, we will highlight some areas where further research is needed.

The study of both cellular and acellular components of the lower respiratory tract is performed using BAL, a minimally invasive bronchoscopic procedure. The technique was first reported in the USA in 1974 by Reynolds and Newball [1] and provided a new way for assessing the pathophysiology of diffuse parenchymal lung diseases without the need for surgical biopsy. Being a safe and informative procedure, there was rapid widespread adoption of the technique for two reasons: 1) the analysis of the different cell populations is used for differential diagnosis between different ILDs, while at the same time excluding infections and malignancy with high confidence; and 2) the collection of both live cells and supernatant allows the development of both cell-based fundamental research and quantification of mediators. These studies have been the source of most of our current knowledge about the pulmonary immune mechanisms during both physiological and pathological conditions.

The number of PubMed papers using BAL for diagnosis and research has been steadily increasing, despite the 40 years that have passed since the first published report. The development of news ways and techniques that could compete with BAL for sample analysis of the lower respiratory tract environment is also increasing.

At present, the clinical and research application of BAL presents some significant challenges. When evaluating the acellular components of the collected fluid, variable dilution of the alveolar lining fluid is to be expected. Concerning the cell population analysis, the initial promises of a straightforward BAL-derived diagnosis for most ILDs did not turn out to be an easy task [2]. Most ILDs are rare or infrequent, complicating the development of adequate clinical trials. A significant number of small studies have shown

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that most diseases do not have a specific cell pattern. This is common in all tests for the diagnosis of ILD. Accordingly, the gold standard for the diagnosis of ILD is a multidisciplinary discussion, where the clinical findings are combined with the results from multiple tests, such as BAL, imaging and biopsies, by a team of physicians that includes a pulmonologist, radiologist and pathologist [3, 4]. It should be noted, however, that in some cases the BAL findings may be very specific and so can directly confirm a particular diagnosis and, thus, can then replace lung biopsy. This is the case for rare ILDs such as pulmonary alveolar proteinosis, Langerhans' cell histiocytosis, diffuse alveolar haemorrhage and ILDs due to mineral dust exposure [5].

Concerning IPF, whereas the role of BAL as a research tool is generally accepted, its place in the clinical management of patients with suspected or confirmed disease is still a matter of discussion. In this chapter we present a review of the most recent advances in both areas, as well as a small discussion on the safety of this technique in this unique population. We will conclude by proposing some areas where further research is required.

Clinical management

The creation of an adequate definition and classification for ILDs has proved to be a large and difficult task, especially for the IIPs. Accordingly, the term IPF has suffered significant changes in its meaning, particularly prior to and after 2000 [6]. These changes render the current interpretation of many previous studies on BAL quite difficult or even impossible. Another issue is the improvement in diagnostic accuracy of alternative tests, especially HRCT [7]. This means that in older studies the diagnostic importance of BAL could be higher, because the imaging findings were not as clear. In this chapter we will focus on the current role of BAL in IPF within the typical circumstances of a specialist ILD centre.

Diagnosis

The recent and widely accepted American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association guidelines on the diagnosis and management of IPF do not recommend the usage of BAL for diagnosis. The committee placed a high value on the additional risk and cost of BAL in patients with IPF and a low value on possible improved specificity of diagnosis [8]. Similarly, the 2012 American Thoracic Society guidelines on clinical utility of BAL in ILD recommend that BAL is not needed when there is a diagnostic HRCT scan, as might be the case in IPF patients with a definite UIP HRCT pattern [5].

The typical BAL cell profile in a patient with IPF shows a predominance of macrophages, with a moderate increase in neutrophils and eosinophils, and normal lymphocyte numbers. This profile is not specific, as other diseases might show the exact same findings, *e.g.* fibrotic NSIP, desquamative interstitial pneumonia or collagen vascular disease-related ILD (table 1) [5]. The main clinical scenario where BAL could be of diagnostic importance is when hypersensitivity pneumonitis is a concern. A seminal study by Ohshimo *et al.* [9] analysed the BAL profile of 74 patients fulfilling the criteria for diagnosis of IPF according to the 2002 guidelines. Six (8%) of these patients had an increase in lymphocytes of >30%. Upon further clinical investigation, all patients were shown to have an alternative diagnosis (hypersensitivity pneumonitis: n=3; NSIP: n=3). The authors argue that a 30% lymphocyte threshold can be used for the exclusion of IPF, thus underlining the importance of BAL

Table 1. BAL changes in IPF and some differential diagnoses

Disease	Macrophages	Neutrophils	Eosinophils	Lymphocytes	Other findings
IPF	Increased	Increased	Increased	Normal	
NSIP	Increased	Normal/ increased	Normal	Increased	
HP	Normal	Normal/ increased	Normal	Very high	Increased total cell count, foamy macrophages, mast cells, plasma cells
DIP/RBILD	Increased	Normal/ increased	Normal/ increased	Normal	Pigmented AM
Sarcoidosis	Normal	Normal/ increased	Normal	Increased	Elevated CD4/CD8 ratio
CVD	Normal/ increased	Elevated	Normal/ elevated	Elevated	

HP: hypersensitivity pneumonitis; DIP: desquamative interstitial pneumonia; RBILD: respiratory bronchiolitis with ILD; CVD: collagen vascular disease; AM: alveolar macrophage.

even in patients with an otherwise clear IPF diagnosis [9]. It is recognised that chronic hypersensitivity pneumonitis and IPF may show identical clinical, imaging and even pathological findings [10]. Some authors have subsequently argued that BAL should only be considered when there is a high local prevalence of hypersensitivity pneumonitis [11]. This is still an active area of discussion. Nevertheless, most authors agree that local experience and expertise will influence the decision on whether or not to perform BAL in patients with suspected IPF [8]. A recent German clinical practice IPF cohort study reported the use of BAL in 310 out of 502 patients, confirming frequent use of BAL in European IPF patients [12]. Some authors have argued that the guidelines on the diagnosis of IPF should make different recommendations for the cases when the clinical and HRCT findings are typical of IPF (definite IPF) *versus* the cases where clinical and imaging findings do not allow a confident diagnosis [13]. In patients with an unclear history and/or imaging findings for IPF, the performance of BAL is more accepted. A cultural difference on the use of BAL between Europe and USA is also acknowledged [13]. For discussion and guidelines on the performance and interpretation of BAL, readers are referred to the updated and comprehensive guidelines on this subject published in 2012 by the American Thoracic Society [5].

Prognosis

Recent studies have not found a consistent benefit of BAL for the evaluation of the prognosis of an individual patient. One study found a correlation between BAL lymphocytosis and survival in patients with fibrotic IIPs [14], while another found increased mortality in patients with increased neutrophils [15]. A third study found no significant associations [16]. There have been no studies on prediction of response to the currently available anti-fibrotic therapies. One interesting finding is the association between chronic aspiration and IPF [17]. Several studies have evaluated the utility of BAL pepsin for identification of chronic aspiration [18]. It is currently unknown whether the quantification of pepsin in the BAL of IPF patients could predict the response to anti-acid therapy.

In conclusion, most authors currently agree that there is no role for BAL in the assessment of prognosis in IPF patients [5].

Exacerbations

Patients with IPF are at risk of episodes of clinical deterioration called acute exacerbations following the exclusion of a number of other causes besides the disease itself. The generally accepted diagnostic criteria for acute exacerbations of IPF require the use of BAL for the exclusion of infection [19]. Some authors have argued that this could lead to unnecessary risks and proposed an algorithm where BAL is only performed in a subset of these patients [20]. The cellular BAL analysis from patients with acute exacerbations of IPF usually shows increased neutrophils [21, 22]. It is important to remember that BAL can be valuable in other causes of deterioration in a patient with IPF, such as drug toxicity and possibly gastric aspiration [20]. In conclusion, BAL should be considered for IPF patients with disease worsening, although a proportion will not need it or tolerate it.

Research tool

The main advantages for using BAL as a research tool are its safety, broad availability and capacity to reflect the global pathophysiology of normal or diseased lung. Although BAL was classically used in the study of parenchymal disorders, its application in airway diseases such as asthma and COPD has been increasing, further strengthening the importance of this procedure [23]. Since BAL recovers both live cells and fluid with soluble mediators, these aspects can be mutually studied in each subject. The broad usage of BAL for clinical reasons means that part of the recovered fluid and cells can be preserved for research, without causing further risk or discomfort to the patient. A final advantage is that BAL can be performed serially, reflecting change with time, although this is not normally performed in clinical practice [23].

Researchers are also faced with some problems when using BAL. Safety should always be a concern as the procedure is not completely free of risk. Secondly, there are significant variations between centres regarding the technique, despite published recommendations for homogenisation [5]. Finally, when studying soluble components, a dilution of the epithelial lining fluid is expected. For the precise quantification of any mediator, this dilution factor should be known. However, different methods have been used [24] and an accurate estimation of the dilution has never been accepted. Published international guidelines have recommended the use of non-corrected quantifications [25]. This limitation has probably prevented the use of BAL quantification methods in clinical practice.

Some of the areas where BAL has been instrumental in IPF research include the study of disease pathophysiology, disease subtypes and response to therapy. Concerning pathophysiology, studies in BAL cells and mediators were helpful in showing IPF to be a fibroblast driven, noninflammatory fibrotic process [26]. Many of the recent discoveries on the importance of microorganisms in the pathogenesis of IPF are also based on BAL [27]. Concerning disease subtypes, Selman *et al.* [28] studied rapid IPF progressors and showed increased BAL concentrations of the A2B adenosine receptor and activated matrix metalloproteinase-9. Finally, the preclinical and clinical development of both pirfenidone and nintedanib benefited from studies using BAL [29, 30].

Safety

BAL is a minimally invasive technique with a favourable risk/benefit ratio. The contraindications and risk factors for complications from BAL are the same as for bronchoscopy. The rate of complications varies from 0% to 2.3%. Post-procedure fever is expected to occur in 3–30% of patients. Its frequency is correlated to total volume used and the mechanism is local release of inflammatory mediators. A transient loss of lung function (possibly leading to hypoxaemia and intubation in severe patients) [31], and radiological infiltrates can also be seen (figure 1). Patients with airway hyperreactivity can develop wheezing, and recommendations for the use of BAL in asthmatic patients are available [32].

However, the main concern when considering BAL in IPF patients is BAL-induced acute exacerbation. A recent Japanese cohort study evaluated the risk of acute exacerbations of IPF following BAL and found significantly increased risk in the 30 days post-procedure. Importantly, none of the four reported events occurred after a first (diagnostic) BAL. The authors also reviewed all the previous reports of BAL-induced acute exacerbations of IPF [33]. A total of 12 cases were described. Most patients had functional impairment and peripheral inflammation before BAL [33].

In conclusion, BAL is a generally safe procedure in IPF patients. The small number of BAL-induced exacerbations that have been described should not deter clinicians from performing this important procedure when indicated.

Alternatives

Although BAL is considered a safe and minimally invasive procedure, several research groups have tried to develop techniques that offer similar information in a less invasive or safer way. Some of these technologies are induced sputum, exhaled breath condensate and bronchoscopic microprobe collection of pulmonary epithelial lining fluid.

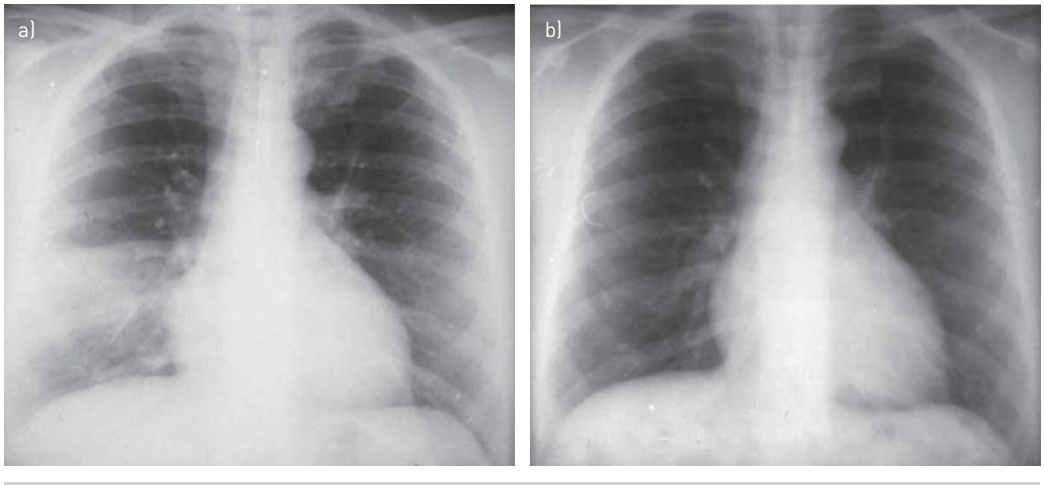

Figure 1. Chest radiograph showing a transient segmental infiltrate in the right middle lobe a) directly following BAL and b) 24 h after BAL.

Induced sputum has been widely used for the study of airways disorders. The technique involves the nebulisation of saline in isotonic or hypertonic concentrations, leading to increased production of sputum that can then be collected and analysed. Similar to BAL, both the cellular and acellular components of induced sputum can be studied. The advantages are a reduced invasiveness with increased possibility for developing serial studies, as well as less need for technology and expertise. The main disadvantage is that induced sputum studies a different component from BAL, since the sputum mostly reflects airway changes. It is generally accepted that induced sputum will not replace BAL in the clinical management and research of IPF, although some authors argue that both methods might be complementary. All in all, the data on induced sputum in IPF is still scarce and uncertain [34, 35].

Exhaled breath condensate is a completely noninvasive method for evaluating the exhaled non-volatile components of the epithelial lining fluid. The technique is quite simple and consists of directing the subject's exhaled breath over a cooled condenser system. Within a few minutes of calm breathing, a small quantity of condensate can be collected for analysis. The procedure can be safely used in children, the elderly and patients of any severity. This method allows for only non-cellular studies. The main use for exhaled breath condensate in IPF is the development of noninvasive biomarkers. The main disadvantage of the method is the extensive dilution of the solutes. This requires extremely sensitive quantification techniques and presently unavailable robust and accepted dilution markers. There is also a problem with significant methodological heterogeneity between different centres and studies [36].

Bronchoscopic microprobe sampling uses a small probe with an absorptive tip, which is introduced through the working channel of a bronchoscope to collect undiluted epithelial lining fluid from the tracheobronchial tree. The technique has been applied in studies on COPD, lung cancer, acute respiratory distress syndrome and epithelial lining fluid drug penetration. Some of the advantages of bronchoscopic microprobe over BAL are the lack of dilution, precise location of sampling and less contamination with serum proteins. The high concentration of proteins on bronchoscopic microprobe samples makes this technique particularly promising for proteomic studies. A non-bronchoscopic method has also been applied in rabbits [37].

Further research

Worldwide, many centres have been using BAL for the study of patients with suspected IPF for decades. It is therefore surprising that there is still significant discussion around the simple recommendation for its use in the diagnosis of IPF. This reflects the major need for large-scale, well-conducted studies on the benefits of BAL for the clinical management of suspected IPF patients. An example of the successful application of such studies can be found with transbronchial cryobiopsy, a new technique that can be useful in the diagnosis of IPF [38]. An equally important consideration is the interest of novel recommendations that clearly distinguish between those cases with typical features from the nondiagnostic clinical and imaging characteristics [13].

Another future clinical application of BAL in IPF is the development of biomarkers for diagnosis, evolution and response to therapy. However, biomarkers should be noninvasive and cheap, so the peripheral blood would be a better source for clinical application. We argue that BAL studies could facilitate the development phase, followed later by studies of blood [39].

The use of omics technologies, comprehensively studying a related set of biological molecules in an unbiased fashion, has been a valuable source of information on the causes, pathophysiology and treatment for human disease. Concerning BAL in IPF, the use of protein studies (proteomics) is particularly promising. In a recent study, FOSTER *et al.* [40] identified and validated pro-fibrotic cytokines in BALF of IPF patients. Marker profiling and targeted quantitation of soluble markers through proteomic approach can give insight into IPF pathology and response to therapy.

Finally, we propose greater use of BAL as a source of live cells for basic research on new therapeutic targets. The currently available animal models of IPF have significant limitations, including major differences in pathogenesis and inter-species differences in pharmacological targets when compared to human IPF [41]. BAL cells collected from IPF patients under normal clinical management can be used as a source of identification for modulators of fibrogenesis [40] and eventually for identifying new targets for therapy.

References

1. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974; 84: 559–573.
2. Meyer KC, Raghu G. Bronchoalveolar lavage for the evaluation of interstitial lung disease: is it clinically useful? *Eur Respir J* 2011; 38: 761–769.
3. Maher TM. A clinical approach to diffuse parenchymal lung disease. *Immunol Allergy Clin North Am* 2012; 32: 453–472.
4. Tomassetti S, Piciucchi S, Tantalocco P, *et al.* The multidisciplinary approach in the diagnosis of idiopathic pulmonary fibrosis: a patient case-based review. *Eur Respir Rev* 2015; 24: 69–77.
5. Meyer KC, Raghu G, Baughman RP, *et al.* An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med* 2012; 185: 1004–1014.
6. Travis WD, Costabel U, Hansell DM, *et al.* An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013; 188: 733–748.
7. Walsh SL, Hansell DM. High-resolution CT of interstitial lung disease: a continuous evolution. *Semin Respir Crit Care Med* 2014; 35: 129–144.
8. Raghu G, Collard HR, Egan JJ, *et al.* An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; 183: 788–824.
9. 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.
10. Cordeiro CR, Alfaro TM, Freitas S. Clinical case: differential diagnosis of idiopathic pulmonary fibrosis. *BMC Res Notes* 2013; 6: Suppl. 1, S1.
11. Kinder BW, Wells AU. The art and science of diagnosing interstitial lung diseases. *Am J Respir Crit Care Med* 2009; 179: 974–975.
12. Behr J, Kreuter M, Hoeper MM, *et al.* Management of patients with idiopathic pulmonary fibrosis in clinical practice: the INSIGHTS-IPF registry. *Eur Respir J* 2015; 46: 186–196.
13. Wells AU. Managing diagnostic procedures in idiopathic pulmonary fibrosis. *Eur Respir Rev* 2013; 22: 158–162.
14. Ryu YJ, Chung MP, Han J, *et al.* Bronchoalveolar lavage in fibrotic idiopathic interstitial pneumonias. *Respir Med* 2007; 101: 655–660.
15. Kinder BW, Brown KK, Schwarz MI, *et al.* Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 2008; 133: 226–232.
16. 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.
17. Raghu G, Meyer KC. Silent gastro-oesophageal reflux and microaspiration in IPF: mounting evidence for anti-reflux therapy? *Eur Respir J* 2012; 39: 242–245.
18. Meyer KC. Gastroesophageal reflux and lung disease. *Expert Rev Respir Med* 2015; 9: 383–385.
19. Collard HR, Moore BB, Flaherty KR Jr, *et al.* Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007; 176: 636–643.

20. Petrosyan F, Culver DA, Reddy AJ. Role of bronchoalveolar lavage in the diagnosis of acute exacerbations of idiopathic pulmonary fibrosis: a retrospective study. *BMC Pulm Med* 2015; 15: 70.
21. Ambrosini V, Cancellieri A, Chilosi M, *et al.* Acute exacerbation of idiopathic pulmonary fibrosis: report of a series. *Eur Respir J* 2003; 22: 821–826.
22. Parambil JG, Myers JL, Ryu JH. Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy. *Chest* 2005; 128: 3310–3315.
23. Rose AS, Knox KS. Bronchoalveolar lavage as a research tool. *Semin Respir Crit Care Med* 2007; 28: 561–573.
24. Haslam PL, Baughman RP. Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. *Eur Respir J* 1999; 14: 245–248.
25. Baughman RP. Technical aspects of bronchoalveolar lavage: recommendations for a standard procedure. *Semin Respir Crit Care Med* 2007; 28: 475–485.
26. Buckley S, Shi W, Xu W, *et al.* Increased alveolar soluble annexin V promotes lung inflammation and fibrosis. *Eur Respir J* 2015; 46: 1417–1429.
27. Molyneaux PL, Cox MJ, Willis-Owen SA, *et al.* The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2014; 190: 906–913.
28. Selman M, Carrillo G, Estrada A, *et al.* Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One* 2007; 2: e482.
29. Iyer SN, Gurujeyalakshmi G, Giri SN. Effects of pirfenidone on transforming growth factor-β gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis. *J Pharmacol Exp Ther* 1999; 291: 367–373.
30. Wollin L, Maillet I, Quesniaux V, *et al.* Antifibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. *J Pharmacol Exp Ther* 2014; 349: 209–220.
31. Bonella F, Ohshimo S, Bauer P, *et al.* Bronchoalveolar lavage. *In*: Strausz J Bolliger CT, eds. Interventional Pulmonology (ERS Monograph). Sheffield, European Respiratory Society, 2010; 48: pp. 59–72.
32. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1985; 132: 180–182.
33. Sakamoto K, Taniguchi H, Kondoh Y, *et al.* Acute exacerbation of IPF following diagnostic bronchoalveolar lavage procedures. *Respir Med* 2012; 106: 436–442.
34. Araujo L, Beltrao M, Palmares C, *et al.* Induced sputum in interstitial lung diseases – a pilot study. *Rev Port Pneumol* 2013; 19: 53–58.
35. Economidou F, Samara KD, Antoniou KM, *et al.* Induced sputum in interstitial lung diseases: novel insights in the diagnosis, evaluation and research. *Respiration* 2009; 77: 351–358.
36. Dompeling E, Rosias PPR, Jobsis Q. Exhaled breath condensate sample collection: standards and open issues. *In*: Horvath I de Jongste JC, eds. Exhaled Biomarkers (ERS Monograph). Sheffield, European Respiratory Society, 2010; 49: pp. 152–161.
37. Franciosi L, Govorukhina N, Fusetti F, *et al.* Proteomic analysis of human epithelial lining fluid by microfluidics-based nanoLC-MS/MS: a feasibility study. *Electrophoresis* 2013; 34: 2683–2694.
38. Tomassetti S, Wells AU, Costabel U, *et al.* Bronchoscopic lung cryobiopsy increases diagnostic confidence in the multidisciplinary diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2015; [In press; DOI: 10.1164/rccm.201504-0711OC].
39. O'Riordan TG, Smith V, Raghu G. Development of novel agents for idiopathic pulmonary fibrosis: progress in target selection and clinical trial design. *Chest* 2015; 148: 1083–1092.
40. Foster MW, Morrison LD, Todd JL, *et al.* Quantitative proteomics of bronchoalveolar lavage fluid in idiopathic pulmonary fibrosis. *J Proteome Res* 2015; 14: 1238–1249.
41. Degryse AL, Lawson WE. Progress toward improving animal models for idiopathic pulmonary fibrosis. *Am J Med Sci* 2011; 341: 444–449.

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