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Editorial

Bronchoalveolar Lavage: A Forgotten Tool!

Since its introduction by Professor Shigeto Ikeda in 1964, the use of flexible fiberoptic bronchoscopy (FOB) is expanding and it is considered to be one the most important breakthrough in diagnostic pulmonology. Bronchoalveolar lavage (BAL) is a specialised technique, described by Reynolds and Newball[1] in 1974, which consists instillation of small quantity of saline directly into distal airways and recovering the aspirate through FOB. This technique allows recovery of both cellular and non-cellular components from the epithelial surface, which are representative of inflammatory and immune system of the entire lower respiratory tract. For the last three decades, BAL has been extensively used for evaluation of various pulmonary conditions including infectious, inflammatory and malignant diseases. However, after years of use it has been realised that it has many limitations. This is true for almost all the modalities that with time only one can learn about their limitations. However, it is clinician's duty to find out the best use of any new tool keeping the limitations in foresight. This applies to BAL also and we argue that BAL fluid analysis is still a useful diagnostic tool, if it is interpreted in context of clinical presentation, radiographic imaging studies and other pertinent testing. Importantly, one must understand its technical aspects, limitations and information which can be useful in clinical practice.[1-4]

It has been observed that the technique used to obtain BAL differs from centre to centre over the world and these differences may alter final results. Therefore, efforts have been put to minimise the limitations associated with technique involved during BAL. Detailed recommendations and guidelines both for technique of bronchoscopy and analysis of BAL fluid has been developed and published to standardise the procedure so that limitations related to different methods followed by various bronchoscopists all over the world can be minimised.[4,5]

Keeping the afore-mentioned limitations in mind, BAL can be used as a minimally invasive diagnostic technique that yields insights into immunologic, inflammatory, and infectious processes occurring at the alveolar level. Many a times it is diagnostic for lung conditions. Even if it is not diagnostic, BAL can help in revising the differential diagnoses. In diffuse infiltrative lung disease, differential cell counts and CD4+:CD8+ T-lymphocyte ratio obtained from cyto-centrifugation of BAL fluid can provide important information. When BAL findings are combined with high resolution computed tomography and other clinical data, it can be diagnostic of specific interstitial lung disease (ILD).[6] Total and differential cell counts of BAL may be used as useful parameters which help in making diagnosis and predicting and monitoring response to therapy in these patients. Notably, usual interstitial pneumonia is frequently associated with elevated BAL neutrophils, whereas non-specific interstitial pneumonia shows raised neutrophils and lymphocytes. The BAL lymphocytosis is found to be a good predictor of response to corticosteroids. Raised eosinophils were associated with poor response, whereas neutrophil counts have no use in predicting response to this therapy. The BAL CD4+ T-lymphocyte subset and CD4+:CD8+ T-lymphocyte ratio are frequently used to diagnose clinically active pulmonary sarcoidosis; and an elevated CD4+:CD8+ T-lymphocyte ratio of 3.5 or more has been shown to be fairly specific for sarcoidosis.[7] On the contrary, decreased CD4+:CD8+ T-lymphocyte ratio is seen in hypersensitivity pneumonitis, drug-induced lung disease, cryptogenic organising pneumonia, eosinophilic pneumonia, and idiopathic pulmonary fibrosis. The CD4+:CD8+ T-lymphocyte ratio has not been found of much help in the diagnosis of non-sarcoid ILD, and its routine examination is not cost effective too. Similarly, BAL flow cytometry showing increased (>5% of total cells) CD1a+ cells are consistent with pulmonary Langerhans' cell histiocytosis (PLCH) in clinical and radiologically suspected cases.[8] Notably, BAL fluid analysis can be highly suggestive or even virtually diagnostic of specific ILD entities in the appropriate clinical settings, like sarcoidosis, PLCH, diffuse alveolar haemorrhage, alveolar proteinosis, etc. More importantly, BAL is most useful in excluding other diagnoses, particularly infections. The BAL can identify the nature of mineral dust and has been used for evaluation of various occupational lung diseases, like asbestosis, silicosis, coal worker's pneumoconiosis and berylliosis, etc.[9] In workers who are at the risk of developing asbestosis, BAL fluid shows multifold increase in cellularity with mild to moderate increase in neutrophils, eosinophils, and selected subgroup of lymphocytes. Demonstration of asbestos bodies in BAL may alert the physicians that the diffuse lung disease is not idiopathic and favours the diagnosis of asbestosis. Interestingly, there is good correlation between level of asbestos bodies present in BAL and lung tissue, therefore, can be used to quantify asbestos in lung.[9] In berylliosis, BAL fluid shows markedly increased cell count, two- to three-fold increase in macrophages, and a predominance of CD4+ T-lymphocytes. Demonstration of sensitised T-cells in BAL fluid has high sensitivity and specificity for the diagnosis of berylliosis.

Similarly, BAL has shown undisputable benefits in the diagnosis of lung infections in immuncompetent as well as immunocompromised patients. Uncentrifuged BAL can be used for quantitative bacterial culture and centrifuged BAL specimens (re-suspended cell pellets)

for culture of viruses and atypical pathogens like, *Legionella*, *Mycoplasma*, and *Chlamydia*, mycobacteria and fungi. Additionally, cyto-centrifuged specimens (cytospin preparations) can be stained to detect the presence of bacteria, *Pneumocystis jiroveci*, mycobacteria, or fungi.[10] Interestingly, BAL fluid can be used for rapid diagnostic techniques using immunofluorescent staining with antibodies and/or nucleic acid analysis via polymerase chain reaction (PCR) methods to facilitate more rapid diagnosis than culture techniques. The BAL PCR has an important role in the diagnosis of smear-negative pulmonary tuberculosis. In immunocompromised patients, BAL frequently identifies the offending pathogen in patients showing lung infiltrates on radiological imaging. Importantly, BAL can be performed safely and rapidly, even in patients with significant coagulopathies. It can be combined with transbronchial lung biopsy (TBLB) to increase the yield but there is significant risk of haemorrhage during lung biopsy in patients with coagulopathy. Similarly, BAL has important role in the management of patients with febrile neutropenia and ventilator-associated pneumonia. In lung transplant recipients, FOB is invaluable tool for both to diagnose acute deterioration of graft function as well as for surveillance purposes to rule out infection or rejection. In this situation, BAL is usually combined with TBLB. The BAL fluid is extremely useful for the detection of infection and can be used for culture, special staining or PCR for various pathogens. Total white blood cell counts and differential count, may contribute significantly but must be interpreted carefully. The BAL lymphocytosis may point to acute rejection, viral infection, or obliterative bronchiolitis, increased neutrophils indicate bacterial infection. The BAL fluid can also be used to diagnose chronic rejection via micro-array analysis of cell gene expression[11] or protein analysis by matrix-assisted laserdesorption ionisation time-of-flight (MALDI-TOF) profiles.[12] Currently, role of BAL and TBLB for surveillance purposes to evaluate clinically stable patients following transplant is somewhat controversial, however, newer techniques such as profiling gene expression applied to BAL are promising.[13]

In lung malignancies, BAL can provide useful information regarding final diagnosis. For example, it helps to differentiate bronchoalveolar carcinoma, lymphangitic carcinoma, or lung infiltration by bone-marrow derived malignant cells, all masquerading as ILD on chest radiographic imaging. Some malignancies, like, adenocarcinoma, and tumours with lymphangitic or lepidic growth patterns are more easily diagnosed by BAL where the diagnostic yield may be higher than 80 percent.[14] In haematologic malignancies, BAL cell surface markers and/or PCR can be used to identify B- or T-cell lymphomas. Its diagnostic yield is quite good in secondary diffuse indolent B-cell lymphomas and in primary B-cell lymphomas of mucosa-associated lymphoid tissue type but low in Hodgkin's disease.[14]

As a newer development, the field of genomics and proteomics is rapidly expanding and these technologies are now being exploited to establish gene expression profiles for various specific disease states, including ILD and lower respiratory tract infection. The BAL cells were shown to be right candidates for this analysis. These cutting edge methods may eventually prove extremely useful in making an accurate diagnosis, selecting and implementing effective therapies, monitoring disease activity, and assessing response to therapeutic interventions. With further advancement, there may be hope that the products of genes linked to disease pathogenesis can be identified and quantitated.[15] The BAL can be used to determine gene and protein expression patterns over time and can potentially identify the key molecules involved in the initiation and progression of the different ILDs, provide an accurate clinical diagnosis of specific ILD without resorting to lung biopsy, and indicate targets for new and effective therapies. Additionally, nucleic acid amplification techniques performed on BAL can provide diagnostic information that surpasses the capabilities of conventional analytic methods used for infectious diseases.[16] Furthermore, a new diagnostic tool (GreeneChipPm, Agilent Technologies, Santa Clara, CA) detecting a broad spectrum of pathogen oligonucleotides (viruses, bacteria, fungi, and parasites) in body fluids rapidly has recently been used for pathogen detection.[17] It holds a great potential to improve the differential diagnosis of lower respiratory tract infection when applied to BAL analysis.

Although BAL has been used for more than 30 years, quite often this specialised technique is omitted because it is supposed to be a cumbersome procedure with limited diagnostic value. However, with experience it takes few additional minutes to perform and the information gathered from it adds to the diagnostic value of FOB. Furthermore, therapeutic BAL where large amount of fluid is used to wash lower airway and is used for the treatment of pulmonary alveolar proteinosis. Therefore, it is suggested that every bronchoscopist should be well conversant with this not so commonly used technique.

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