Chapter 2
Bronchoalveolar Lavage: Tests and Applications

Fabio Midulla, Raffaella Nenna, and Ernst Eber

Abstract Bronchoalveolar lavage (BAL) is a diagnostic procedure used for recovering cellular and non-cellular components of the epithelial lining fluid of the alveolar and bronchial airspaces. Non-bronchoscopic BAL involves the insertion of simple catheters or balloon-type devices through an endotracheal tube, while bronchoscopic BAL involves the instillation and immediate withdrawal of pre-warmed sterile 0.9 % saline solution through the working channel of a flexible bronchoscope, preferably in the middle lobe or the lingula. The parameters measured in BAL fluid (BALF) include the percentage of the instilled normal saline that is recovered as well as various BALF cellular and non-cellular components. BAL is performed for diagnostic, therapeutic, and research purposes. The most important indication for BAL in children is in the work up of infectious diseases. Other indications for BAL include non-specific chronic respiratory symptoms, non-specific radiological findings, and clinical symptoms suggestive of interstitial lung disease. BAL is still considered to be the gold standard for diagnosing chronic pulmonary aspiration. BAL is a well-tolerated and safe procedure; however, on occasion, fever, cough, transient wheezing, and pulmonary infiltrates have been observed, which usually resolve within 24 h.

Keywords Bronchoalveolar lavage • Bronchoscopy • Complications • Epithelial lining fluid • Indications • Reference values • Techniques • Therapeutic applications

Bronchoalveolar lavage (BAL) is a predominantly diagnostic procedure used for recovering cellular and non-cellular components of the epithelial lining fluid (ELF) of the alveolar and bronchial airspaces. The procedure usually consists of the instillation and immediate withdrawal of pre-warmed sterile 0.9 % (normal) saline solution (NSS) through the working channel of a flexible bronchoscope (FB), which has been wedged into a bronchus with a matching diameter.

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Procedures

Current clinical practice utilises two techniques: non-bronchoscopic and bronchoscopic BAL. Non-bronchoscopic BAL involves the insertion of simple catheters or balloon-type devices (size 4–8 French) through an endotracheal tube [1].

Bronchoscopic BAL involves instillation and withdrawal of NSS through the working channel of a flexible bronchoscope. The preferred sites for BAL in diffuse lung diseases are the middle lobe and the lingula because, being the smallest lobes of each lung, they offer better fluid recovery. When lung disease is localised, BAL must target the radiologically or endoscopically identified involved lobe or segment. In patients with cystic fibrosis, samples from multiple sites should be obtained in order to avoid underestimation of the extent of infection [2]. To avoid contamination, BAL must precede any other planned bronchoscopic procedure.

In order to obtain the lavage specimen in the dedicated collection trap, gentle manual or mechanical suction (3.33–13.3 kPa, i.e. 25–100 mmHg) is applied, while maintaining the tip of the bronchoscope wedged into the selected site. The saline utilised for BAL is pre-warmed to body temperature (37 °C) in order to prevent the cough reflex. Flow from the distal tip of the bronchoscope can be observed during fluid instillation.

Three optional methods are currently used for calculating the total amount of sterile NSS for BAL to obtain samples that are representative of the alveolar compartment [3]. Some authors choose to use 2–4 aliquots of equal volume (10 ml per aliquot for children less than 6 years, and 20 ml per aliquot for children over 6 years of age), irrespective of the patient's body weight [4]. Others suggest the use of three aliquots, each consisting of 1 ml/kg body weight for children weighing up to 20 kg, and three 20 ml aliquots for heavier children. Lastly, de Blic et al. [5] have recommended that the amount of instilled NSS be adjusted up to a maximum volume of 10 % of the child's functional residual capacity (FRC).

In general, BAL is considered technically acceptable if more than 40 % of the total NSS instilled is recovered, and the lavage fluid (except for the first sample) contains few epithelial cells.

Processing of Bronchoalveolar Lavage Fluid

To optimise cell viability, BAL fluid (BALF) must be kept at 4 °C until analysed. BALF specimens should be processed as soon as possible. The variables measured in the BALF include the percentage of fluid recovered (as compared to the amount of NSS instilled) as well as various cellular and non-cellular components. The first unfiltered BALF aliquot is usually processed separately for microbiological studies. While BALF is centrifuged for direct detection of bacteria, viruses, and fungi and to culture fungi, protozoa, and viruses, it is not centrifuged to culture bacteria. The rest of the aliquots are filtered through sterile gauze to remove mucus; then they are pooled and submitted for cytological studies and analysis of the BALF solutes.

BALF can be prepared in two ways: (a) by obtaining cytospin preparations of the whole BALF, and (b) by re-suspension of the specimen in a small amount of medium which is then centrifuged. At least four slides should be prepared for each patient and we recommend storing one or two slides for research purposes. The number of cells per ml in the recovered BALF is counted with a cytometer on whole BALF specimens stained with trypan blue, or with a cytoscan. Alternatively, the slides can be stained with May-Grünwald, Giemsa, or Diff-Quick stains for the evaluation of differential cell counts and cellular morphological features. In particular clinical settings, the slides can also be prepared with specific stains, e.g. Oil Red O stain to detect lipid-laden macrophages, iron stain to identify iron-positive macrophages in patients with alveolar haemorrhage, and periodic acid-Schiff (PAS) to identify glycogen.

Materials for the evaluation of non-cellular components must be obtained from the supernatant after centrifugation.

The composition of the BALF can be influenced by several technical factors including: site of lavage, fluid pH, temperature and volume of instilled NSS, number of aliquots, size of bronchoscope, dwell time, and suction pressure. Another important precautionary measure is to keep the samples in anaerobic transport media that contain reducing agents in order to avoid air exposure that destroys anaerobic bacteria.

Reference Values

Mean BALF total cell count ranges from 10.3 to 59.9×10^4 cells/ml, with a mean of 81.2–90 % for macrophages, 8.7–16.2 % for lymphocytes, 1.2–5.5 % for neutrophils, and 0.2–0.4 % for eosinophils, respectively [6]. The predominant cells, regardless of the child's age, are macrophages, followed by lymphocytes (Table 2.1).

Normal values of BALF lymphocyte subsets in children resemble those found in healthy adults, except for the CD4/CD8 ratio, which is often lower in children, possibly because children frequently suffer from viral infections [7, 8].

The concentration of serum-derived proteins is higher in children than in adults, whereas locally produced mediators do not differ (Tables 2.2 and 2.3). Surfactant phospholipid concentrations are higher in 3–8 year old than in older children, whereas surfactant protein concentrations are independent of the child's age.

Indications for Bronchoalveolar Lavage

BAL is performed for diagnostic, therapeutic and research applications. The most important indication for BAL in children is in the work-up of infectious diseases. BAL can be done in both immunocompromised (lung transplant, HIV infection, chemotherapy) and immunocompetent children (chronic pneumonia, tuberculosis, cystic fibrosis). Other indications for BAL include non-specific chronic respiratory symptoms, non-specific radiological findings, and clinical symptoms suggestive of chronic interstitial lung disease (CILD; diffuse parenchymal lung disease, DPLD).

Table 2.1 Total and differential cell counts in bronchoalveolar lavage fluid (BALF) from control children

	Midulla et al. [20]	
Number of patients	16	
Age range	2–32 m	
Sedation	LA	
Number of aliquots	2	
Lavage volume	20 ml	
BALF recovered %	Mean ± SD	43.1 ± 12.2
	Median	42.5
	Range	20–65
Cell count (10^4 cells · ml^{-1})	Mean ± SD	59.9 ± 8.2
	Median	51
	Range	20–130
AM %	Mean ± SD	86 ± 7.8
	Median	87
	Range	71–98
Lym %	Mean ± SD	8.7 ± 5.8
	Median	7
	Range	2–22
Neu %	Mean ± SD	5.5 ± 4.8
	Median	3.5
	Range	0–17
Eos %	Mean ± SD	0.2 ± 0.3
	Median	0
	Range	0–1

m months, *LA* local anaesthesia, *AM* alveolar macrophages, *Lym* lymphocytes, *Neu* neutrophils, *Eos* eosinophils

Table 2.2 Concentration ($mg\ L^{-1}$) of serum-derived proteins in bronchoalveolar lavage fluid (BALF) from control children

		Midulla et al. [20]
Number of patients		7
Age		1–3 y
Total protein	Mean ± SD	108 ± 39
	Median	67
	Range	44–336
Albumin	Mean ± SD	58 ± 26
	Median	29
	Range	14–210

y years

Table 2.3 Concentration ($mg\ L^{-1}$) of locally produced mediators in bronchoalveolar lavage fluid (BALF) from control children

		Midulla et al. [20]
Number of patients		7
	Age	1–3 y
Fibronectin	Mean ± SD	172 ± 83
	Median	80
	Range	25–640
Hyaluronic acid	Mean ± SD	26 ± 5
	Median	18
	Range	16–45

y years

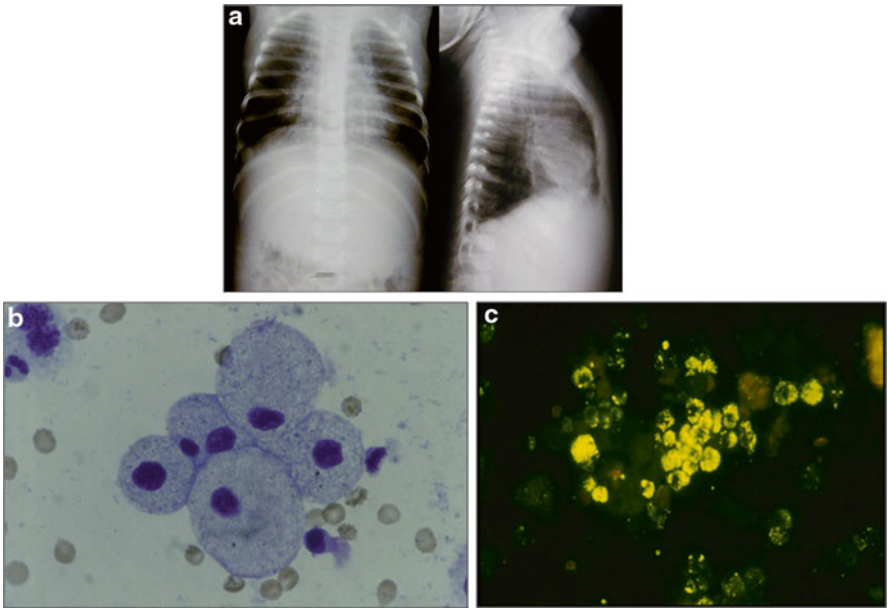

Fig. 2.1 A child with interstitial pneumonia. (**a**) chest X-ray, (**b**) bronchoalveolar lavage cytology showing several alveolar macrophages with foamy cytoplasm (May-Grünwald Giemsa stain, ×100), (**c**) positive direct immunofluorescence for cytomegalovirus antigens

BAL results should be analysed carefully; in fact, BAL is diagnostic only when pathogens not usually found in the lung are recovered, such as *Pneumocystis jirovecii*, *Toxoplasma gondii*, *Strongyloides stercoralis*, *Legionella pneumophila*, *Histoplasma capsulatum*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, influenza virus, and respiratory syncytial virus. The isolation of herpes simplex virus, cytomegalovirus, Aspergillus, *Candida albicans*, Cryptococcus, and atypical mycobacteria from BALF is not diagnostic but may contribute to diagnosis and management of infectious diseases (Fig. 2.1). The presence of equal to or more than 10^4 colony-forming units/ml BALF will identify patients with bacterial pneumonia with reasonable accuracy. Hence, when evaluating the microbiological results, the physician must take into account the underlying disease and the overall clinical picture.

The role of BAL in the diagnosis of lung infection in immunocompetent children is controversial. Bronchoscopy including BAL as invasive procedure is hardly ever justified as a first step in the diagnosis of primary respiratory infection in otherwise healthy children, but should be reserved for patients with atypical manifestations. BAL may be a useful tool in patients with chronic pneumonia, tuberculosis, and cystic fibrosis. In the latter, BAL has an important role in detecting respiratory pathogens and inflammation, especially in young children who are unable to expectorate or in those who fail to improve after therapy [2].

CILD is a heterogeneous group of disorders characterised by typical radiological findings, restrictive lung disease, and inflammation of the pulmonary interstitium [9].

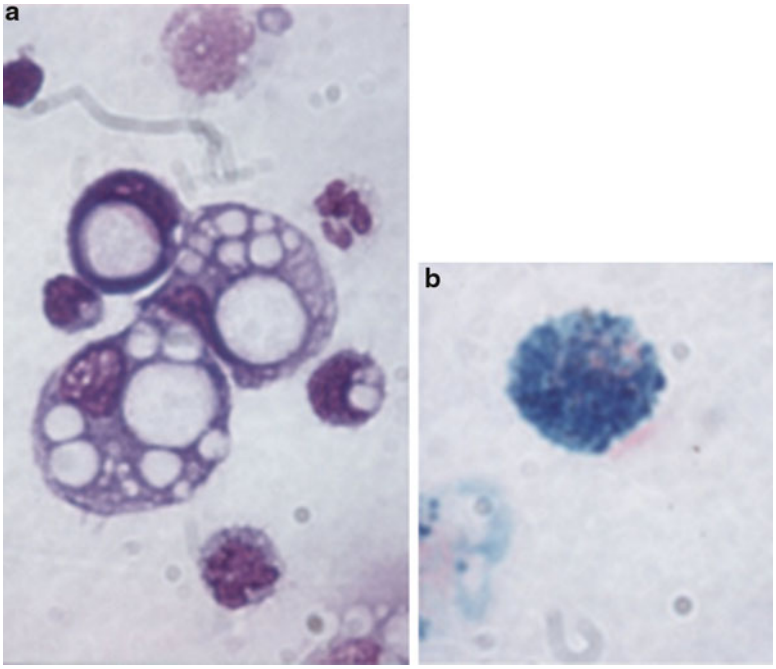

Fig. 2.2 (**a**) Lipoid pneumonia. Bronchoalveolar lavage fluid cytology showing vacuolated alveolar macrophages (May-Grünwald Giemsa stain, ×100). (**b**) Haemosiderin-laden alveolar macrophages in the bronchoalveolar lavage fluid of a patient with alveolar haemorrhage (Prussian blue staining, ×100)

In these patients, BAL may have an important role in reaching or confirming a specific diagnosis, in characterising the alveolitis, and in monitoring the patient during treatment and follow-up [10].

In addition, BALF findings usually provide a specific diagnosis in children with alveolar proteinosis, chronic lipoid pneumonia (Fig. 2.2a), pulmonary histiocytosis, pulmonary haemorrhage (Fig. 2.2b), and pulmonary microlithiasis.

With BALF analysis, three different forms of alveolitis can be identified: lymphocytic, neutrophilic, and eosinophilic (Fig. 2.3):

1. When patients present with clinical manifestations typical of sarcoidosis, a high percentage of lymphocytes (more than 30 %) with predominating CD4 T-cells in the BALF is strongly suggestive, although not definitively confirmatory, of the diagnosis [11].

   Hypersensitivity pneumonitis typically causes lymphocytic alveolitis with the BALF containing predominantly CD8 T-lymphocytes. Similarly, in children with histiocytosis X, or with interstitial lung disease related to collagen disease, or in cryptogenic organising pneumonia (COP; previously termed bronchiolitis obliterans and organising pneumonia—BOOP) the predominant cells are CD8 T-lymphocytes.

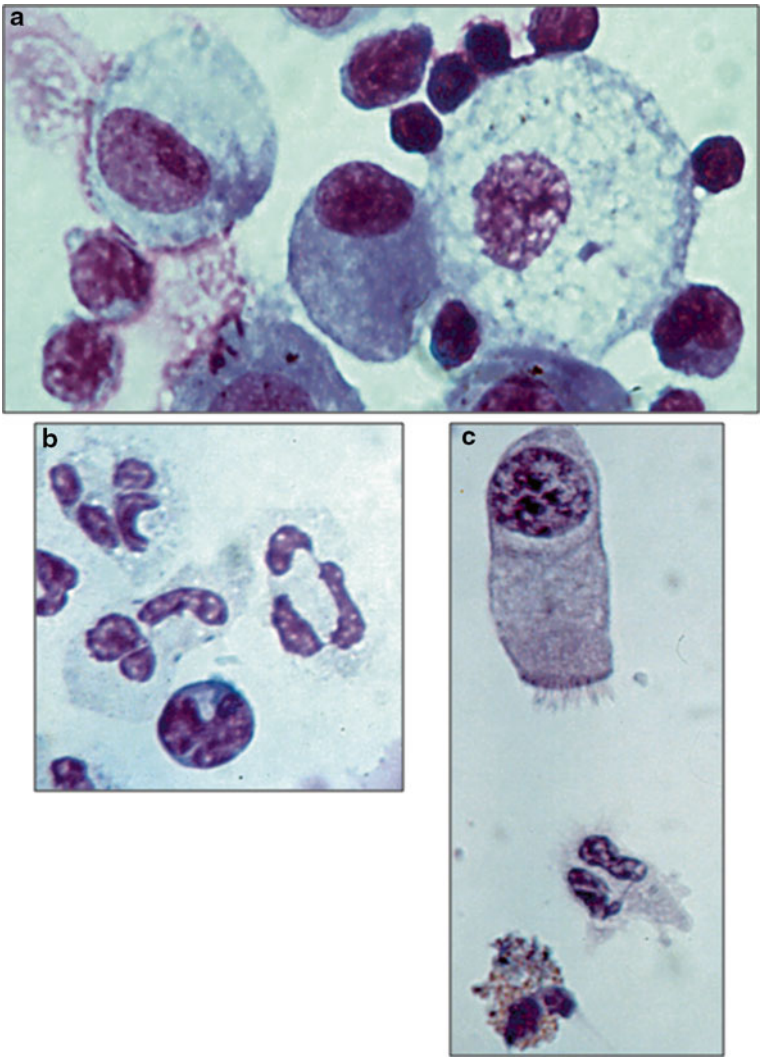

Fig. 2.3 Bronchoalveolar lavage fluid cytology features in (**a**) lymphocytic, (**b**) neutrophilic, and (**c**) eosinophilic alveolitis (May-Grünwald Giemsa stain, ×100)

2. A neutrophilic alveolitis is usually found in idiopathic pulmonary fibrosis and in COP. The histological features are accumulation of macrophages accompanied by mild chronic interstitial pneumonia and, at the worst, mild interstitial fibrosis.
3. Patients with eosinophilic alveolitis or interstitial lung disease always show a predominance of eosinophils in BALF with focal eosinophilic abscesses. The aetiology of this condition often remains elusive; a number of causes (e.g. drug reactions, fungi, parasites, and vapour inhalation) have been described.

BAL is still considered to be the gold standard for the diagnosis of chronic pulmonary aspiration (CPA), i.e. the repeated passage of food material, gastric refluxate, or saliva into the subglottic airways causing chronic or recurrent respiratory symptoms [12]. BAL remains the procedure of choice to diagnose CPA by determining the lipid-laden macrophage (LLM) index [13] and by measuring gastric pepsin concentrations [14, 15]. The LLM index can be calculated by assigning each LLM a score that ranges from 0 to 4 according to the amount of cytoplasmic lipid and scoring one hundred consecutive alveolar macrophages; thus, the highest possible score (LLM index) is 400. A LLM index of more than 100 is considered positive for aspiration [13]. However, the LLM index has certain limitations such as a lack of reproducibility, the inability to differentiate between exogenous and endogenous lipids, and the false-positive results that it may yield in patients with lung disease unrelated to aspiration or even in healthy children [16, 17]. LLM may also be observed in cases of fat embolism [18] and endogenous lipoid pneumonia [19]. Tracheal pepsin has also been used as a marker of reflux aspiration. Pepsin detection in the BALF has been shown to have high sensitivity and specificity values for reflux-related pulmonary aspiration. Unfortunately, pepsin detection is still possible only with "home-made" assays, and its use is strictly limited to the diagnosis of gastric reflux-related aspiration.

Therapeutic Applications

BAL has a major role in the therapy of certain lung diseases, in the form of total lung lavage or mucus plug removal. In particular, children with persistent and massive atelectasis can successfully undergo selective lavage, usually with sterile saline. Mucolytics including DNase and *N*-acetylcysteine as well as exogenous surfactant to help prevent reabsorption atelectasis have also been used, but efficacy has not been demonstrated for these.

Complications

BAL is a well-tolerated and safe procedure; however, on occasion, fever, cough, transient wheezing, and pulmonary infiltrates have been observed, which usually resolve within 24 h. The most frequent complication is fever; the only treatment needed is antipyretics. In immunocompromised patients antibiotic therapy must be performed for 48 h.

BAL may cause hypoxaemia, hypercapnia, or both. Severe bleeding, bronchial perforation, mediastinal emphysema, pneumothorax, and cardiac arrest are extremely rare. Contraindications to the procedure include bleeding disorders, severe hemoptysis, and severe hypoxemia that persists despite oxygen treatment.

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