The Role of Bronchoalveolar Lavage in the Diagnosis of Bacterial Pneumonia

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Bronchoalveolar lavage (BAL) has become an invaluable diagnostic tool with important clinical implications in both opportunistic infections and the pulmonary pathology of immunologic disease. Until recently, the use of BAL was limited primarily to two areas: the study of interstitial lung diseases and the diagnosis of lung infections by opportunistic microorganisms in severely immunocompromised patients with lung infiltrates. Over the past decade, the use of BAL has been expanded to include the conventional diagnosis of bacterial pneumonia in non-immunocompromised patients. In the past, different clinical studies proposed using BAL to quantify cultures in the sample obtained as a means of increasing the tool's effectiveness. Recent developments have led to a number of newer applications of BAL, such as bronchoscopic BAL, non-bronchoscopic BAL and protected BAL. The most important use of BAL in the non-immunocompromised patient is the diagnosis of pneumonia in the mechanically ventilated patient.

Bronchoalveolar lavage (BAL), first introduced in the 1970s and used initially as an experimental procedure to study the cytologic and humoral components present in the alveolar surface, has become an invaluable diagnostic tool with important clinical implications in both opportunistic infections and the immunologic pulmonary pathologies. Over the past decade, the use of BAL has been expanded to include the conventional diagnosis of bacterial pneumonia in non-immunocompromised patients. This review briefly summarises the main findings on the use of BAL in the diagnosis of bacterial pneumonia and describes several new, recently developed uses for BAL.

History

The use of BAL allows us to explore in vivo the epithelial surface of the lung under normal and pathologic conditions, something which cannot be achieved with other internal organs. The idea of studying the cells and substances present in the alveolar surface through a lavage precedes the appearance of fiberoptic bronchoscopy at the beginning of this century. Jackson (1) introduced a series of modifications to the rigid bronchoscope, creating an inhalation channel that permitted bronchial lavages to be used. For 20 years bronchial lavage was used for therapeutic ends, particularly in patients with bronchiectasis (2). Later, newer designs were developed, which brought better results from the lavage (3). With the appearance of Métras's catheter, it was possible to enlarge the area in which the lavage was used, since it allowed segmentary bronchi to be channelled (4). This catheter enabled the first studies of the immunologic function of alveolar macrophages, which were carried out in human volunteers (5–9). As a result of the introduction of fiberoptic bronchoscopy in the 1960s by Ikeda et al. (10), various experiments were performed using healthy volunteers who were also smokers (11). The findings of Reynolds and Newball (12) served as the basis for numerous studies that analyzed the different cells and substances involved in the inflammatory damage and immunopathogenesis of many lung diseases (13–19).

Bronchoalveolar lavage became popular immediately, mainly due to its easy and repeatable use, its safeness and its ability to explore a large portion of lung tissue. Its value as an exploratory tool for the lung is largely derived from the fact that the

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fluid obtained by means of BAL reproduces exactly the inflammatory changes present in the lung tissue (20). Two international symposiums have approved and recommended the use of BAL in clinical practice (21, 22).

Technical Considerations about the Use of Bronchoalveolar Lavage

Bronchoalveolar lavage is performed by injecting and re-aspirating sterile saline solution through a suction channel in the fiberoptic bronchoscope, placed in a subsegmentary bronchi. The amount of liquid used for the diagnosis of infectious lung diseases has not been standardised. The BAL Cooperative Group Steering Committee has recommended using 240 ml for evaluation of patients with interstitial lung disease (23). In studies carried out using BAL to obtain bacterial cultures, including from patients under mechanical ventilation, the amount of liquid used varied between 100 and 240 ml (24). According to some studies, at least 120 ml is needed to obtain secretions from the most distal portions of lung subsegments (25). A 5 ml sample of fluid is the minimum recommended for correct microscopic and microbiological processing (26). The effect of dilution on the results of bacterial cultures has not been studied.

Bacterial Pneumonia and Bronchoalveolar Lavage

Until a few years ago, the use of BAL was limited primarily to two main areas: the study of interstitial lung diseases and the diagnosis of lung infections by opportunistic microorganisms or by obligate pathogens, where the contamination of fluid by the oropharynx flora does not represent a diagnostic problem (27). In severely immunocompromised patients with lung infiltrates, BAL is considered a principal diagnostic tool (28). In animal studies BAL was as sensitive as lung biopsy in the detection of *Pneumocystis carinii* (29). Bronchoalveolar lavage is also the most advanced diagnostic tool when used in AIDS patients and in those suspected to be infected with *Pneumocystis carinii* (30). The use of BAL for the diagnosis of bacterial pneumonia, however, has been questioned, since it is believed that samples obtained by BAL will be invariably contaminated by

oropharynx bacteria after their passage through the channel of the fiberoptic bronchoscope in the same way that bronchoscopy samples are contaminated, as shown by the work of Bartlett et al. (31).

Contamination of the fluid by potentially pathogenic microorganisms considerably limits the tool's usefulness in the diagnosis of conventional bacterial pneumonia. In 1987 two studies proposed that cultures of samples obtained by BAL be quantified as a means of increasing the tool's effectiveness. In the first of these, Thorpe et al. (32) researched the usefulness of Gram stain and semiquantified cultures of BAL fluid in a heterogeneous group of 92 hospital patients. Of 15 patients with active bacterial pneumonia, 13 had cultures with counts of 10^5 cfu/ml, and the rest, including controls, patients with pneumonia in resolution and patients with chronic bronchitis, had counts of 10^4 cfu/ml or, in most cases, less than 10^4 cfu/ml. In addition, the Gram stain was closely correlated with results obtained by BAL. In the prospective study of Kahn and Jones (33), 57 immunocompromised patients with infiltrated lungs were studied along with 18 controls by means of fiberoptic bronchoscopy with BAL. The samples taken were processed quantitatively. In the 18 controls, none of whom had evidence of respiratory infection, the presence of more than 1 % squamous epithelial cells suggested contamination of the sample by oropharynx flora. Of the 13 patients with bacterial pneumonia confirmed by other methods, each had less than 1 % squamous cells and at least one microorganism that was isolated at a concentration of 10^5 cfu/ml. One organism cultured from one BAL at a concentration of 10^5 cfu/ml and the presence of 1 % squamous cells resulted in a sensitivity of 88 % and a specificity of 100 %. However, contamination of BAL fluid occurred in 26 % of the cases.

In a more recent study, Kirkpatrick and Bass (34) showed that the BAL fluid could be contaminated by the bacterial oropharynx flora. Samples for quantitative culture were obtained from eight healthy volunteers using a protected specimen brush (PSB), in accordance with the Wimberly technique (35), and BAL. Cultures of samples obtained by BAL were positive in seven of eight cases, while only one sample obtained by PSB was positive. Although the concentration of microorganisms was not significant ($< 10^5$ cfu/ml), the study showed that contamination by the upper airway is common in BAL cultures; the authors attributed the contamination in part to the introduction of lidocaine into the fiberoptic broncho-

scope channel, which had been done prior to the use of BAL.

Bronchoalveolar Lavage in Pneumonia Associated with Mechanical Ventilation

The majority of studies investigating the use of BAL in bacterial pneumonia were carried out in mechanically ventilated patients. Table 1 shows the most representative studies of this type. Until very recently, it was widely accepted that the PSB described by Wimberly et al. (35, 36) was the best way to obtain microorganisms for identification in patients with pneumonia associated with mechanical ventilation and to differentiate between colonisation of the main respiratory tract and distal lung infection. The initial studies by Higuchi et al. (37) and Chastre et al. (38), which correlated PSB cultures with the histological findings and quantitative cultures of lung tissue, stimulated clinical and experimental research in this area.

Since then, numerous studies have proven the diagnostic efficiency of the PSB in pneumonia associated with mechanical ventilation (39–44). In a total of 18 studies evaluating the PSB in 524 patients with mechanical ventilation, the overall sensitivity of the tool was 90 %, and the specificity was 94 % (45). The use of the PSB, however, is not without disadvantages. The percentage of false-positive and false-negative results varies between 10 and 30% (46), particularly in patients with underlying pathologies that favour colonisation of the distal airway (47, 48) and those with mechanical ventilation and prolonged antibiotic therapy (38, 41, 44). The small volume of secretions obtained using the PSB also contributes to these rates (37, 49). In addition, the results of culture tend to be prolonged (requiring > 24 h), and fewer cases of polymicrobial pneumonia are detected (50). Finally, complications of PSB use,

such as pneumothorax and endobronchial haemorrhage, have been described (44). In four recent studies, the efficiency of BAL versus PSB was compared in animals (51) and in patients on mechanical ventilation (52–54). Despite good results achieved by BAL when used in non-intubated patients, its use in mechanically ventilated patients has been more controversial, mainly due to the heterogeneous populations that have been studied and the different methods used.

In the study by Johanson et al. (51), carried out in 35 monkeys with prolonged mechanical ventilation, cultures of endotracheal aspirates, BAL fluid, PSB samples and needle aspirates were compared with the histological findings of lung biopsy. The 'bacterial charge' of the lung was expressed as the 'bacterial index' (calculated by the sum of the logarithmic concentration of individual bacterial species). BAL recovered 74 % of all species present in the lung tissue, compared with 41 % obtained by PSB and 56 % by needle aspiration. The specificity of BAL, however, was slightly inferior to that of the PSB: 8 % of the samples obtained using BAL in animals without pneumonia had significant growth. The authors of the study concluded that the quantitative analysis of samples obtained by BAL reflected the microbiological spectrum of the lung, and thus BAL was judged the most efficient of the three tools studied. BAL was the most sensitive of all three tools, although the specificity of the three methods was similar.

Chastre et al. (52) were the first to compare BAL and the PSB in mechanically ventilated humans. In a group of 21 patients suspected to have pneumonia, all of whom had received antibiotic treatment for more than ten days without the use of the bacterial index or the establishment of different cut-off points, those patients with pneumonia were readily differentiated from those without pneumonia. These authors' study concluded that

Table 1: Diagnostic yield of quantitative cultures of bronchoalveolar lavage fluid in bacterial pneumonia.

Reference (no.)	Year	No. of samples	Sensitivity (%)	Specificity (%)
Kahn and Jones (33)	1987	75	100	100
Johanson et al. (51)	1988	35	74	100
Chastre et al. (52)	1988	21		69
Torres et al. (53)	1989	25	72	71
Gaussorgues et al.* (59)	1989	13	93	89
Rouby et al.* (61)	1993	69	70	69

*Modification of the BAL technique.

the different quantitative cultures of BAL fluid were of little value in identifying lung infections in mechanically ventilated patients. Their finding of 25 % of cells with intracellular organisms was proposed as a marker for pneumonia, with a specificity of 100 %. A year later the same authors (53) compared the microscopic analysis of BAL fluid with the quantitative cultures of PSB specimens in a group of 61 patients with mechanical ventilation and suspected pneumonia. The proportion of cells containing intracellular organisms exceeded 7 % in 12 of 14 patients with pneumonia and in only 2 of those without pneumonia (sensitivity 86 % and specificity 96 %). On the basis of this finding, they established 5 to 7 % of cells with intracellular organisms as a new cut-off point for identification of patients with active pneumonia. The Gram stain of BAL fluid correlated closely with the cultures obtained from the PSB. These authors suggested using both tools in the same endoscopic exploration and concluded that the microscopic analysis of BAL fluid provides rapid identification of individuals colonised with pneumonia-causing organisms, allowing the prompt administration of antibiotic therapy, which subsequently should be modified according to the results of culture of the PSB specimens.

Torres et al. (54) also compared the diagnostic value of quantitative cultures of both PSB specimens and BAL fluid from 25 mechanically ventilated patients with nosocomial pneumonia of short evolution who had also been treated with empiric antibiotics for fewer than 12 hours. The BAL sample was processed by different microbiological methods than those used by other authors (32, 33, 51, 52). The cut-off point for cultures of both PSB specimens and BAL fluid was established at 10^4 cfu/ml. The diagnostic correlation between both tools was excellent. When both tools were combined, sensitivity was 84 %. The sensitivity and specificity of BAL were 59 % and 71 %, respectively, while values for the PSB were 59 % and 86 %. However, although the sensitivity of both tools was identical, the amount of organisms collected was different. In only 14 of 25 patients was there concordance between the type and amount of organisms by both tools. Lastly, Guerra and Baughman (55) studied the benefits of BAL in 60 patients with mechanical ventilation, obtaining a sensitivity of 60 %. Although organism counts in BAL cultures from a group of control patients did not exceed 10^4 cfu/ml, this study lacked a viable reference test and did not allow an exaggeratedly high specificity in comparison with studies done by other authors. Table

1 shows the more significant results achieved using BAL in ventilator-associated pneumonia.

Variations of Bronchoalveolar Lavage Applied to the Diagnosis of Bacterial Pneumonia

The specificity of BAL cultures in diagnosing bacterial pneumonia is limited in part by the contamination by colonising bacteria of the upper airway. Contaminants can be found in significant quantities in up to one-third of the samples obtained (33, 52, 54). Recommendations for avoiding contamination include avoiding suction by the fiberoptic bronchoscope channel before performing BAL, placing the patient in the Trendelenburg position and discarding the first aliquot from the fluid obtained (23). These problems have stimulated the search for new solutions in improving BAL's efficiency while retaining its maximum benefits in diagnostics.

Non-bronchoscopic Bronchoalveolar Lavage. Initially, the use of BAL without the introduction of a fiberoptic bronchoscope in the distal airway was intended for the diagnosis of infection by *Pneumocystis carinii* in AIDS patients (56, 57). Other authors (58–60) later applied non-bronchoscopic BAL (NB-BAL) in patients with ventilator-associated pneumonia. Pugin et al. (60) compared the quantitative cultures obtained by bronchoscopic BAL and non-bronchoscopic BAL in 28 patients with prolonged mechanical ventilation. The results showed a strong correlation between both BAL tools for the type of microorganism isolated. The predominant species recovered was the same in 93 % of the cases, and there were no significant differences in either bacterial index or cellularity. The authors concluded that the good sensitivity and specificity obtained with NB-BAL lies in the fact that the bacterial spectrum of nosocomial pneumonia in mechanically ventilated patients is similar in all lung lobules and in both lungs, even when a more radiographically localised infiltrate exists.

Despite this study and others such as that by Rouby et al. (61), who defined ventilator-associated pneumonia as a diffuse and multifocal process with varying grades of severity, limited data suggest the usefulness of this technique, which is less expensive than fiberoptic bronchoscopy, in clinically unstable patients, particularly when an experienced bronchoscopist is not available. On the other hand, in the event of a local

pulmonary process in the upper lung lobules, it is preferable to obtain samples by fiberoptic bronchoscopy.

Protected Bronchoalveolar Lavage. Protected BAL (P-BAL) is a variation of conventional BAL in which sealed probes or distally protected catheters are used. Protected BAL was designed to avoid contact between the fluid used in BAL and the contaminating microorganisms that may be present in the suction channel of the broncho-scope, recalling the principal employed in the catheter designed by Wimberly et al. (35).

Rouby et al. (62) were the first to describe a protected BAL technique, employing a double catheter (Combicath, Lab Plastimed, France) distally sealed. Once the fiberoptic bronchoscope is inside the bronchial periphery, a mini-BAL with 20 ml of saline serum is instilled, and at least 1 ml is recovered for bacteriologic processing. The value of this new technique was tested in two groups of patients with mechanical ventilation, one control group of 29 patients and another group of 30 patients who died of nosocomial pneumonia that had been confirmed both bacteriologically and histologically. Using qualitative cultures only, the sensitivity of P-BAL was 80 % and the specificity 66 %. Of the 43 microorganisms isolated from lung tissue cultures, 74 % were recovered by the P-BAL. The different methods used to perform P-BAL are shown in Table 2.

Meduri et al. (63) have described a use of the P-BAL in which a double-light, protected, balloon-tipped catheter is employed. Placement of this catheter requires the aid of a fiberoptic broncho-scope. Furthermore, due to its thickness, the catheter needs a work channel of 2.6 mm. Once the fiberoptic bronchoscope is installed in the zone chosen for P-BAL, the distal balloon must be inflated with 1.5 to 2 ml of air, and the distal diaphragm that seals the whole system must be

expelled using 3 ml of sterile saline solution. Protected BAL is performed through a catheter with 5 aliquots of 30 ml of saline solution. The control group comprised 18 patients with neither pneumonia nor mechanical ventilation. The study group included 28 patients with suspected pneumonia, most of whom were intubated and mechanically ventilated. Ninety-one percent of the samples obtained by P-BAL contained < 1 % squamous epithelial cells, and 59 % of the samples from patients without pneumonia showed no growth. Establishing a cut-off point of 10^4 cfu/ml, 1 of every 33 patients without pneumonia had a false-positive result, and 1 of every 13 patients with pneumonia had a false-negative result (positive predictive value and sensitivity, 97 %; negative predictive value, 92 %). Microscopic analysis using Gram stain had a sensitivity and specificity of 92 % and 97 %, respectively. Fluid was not recovered from 2 of the 49 patients included in the study.

Following the development of P-BAL, a curious variation of P-BAL was proposed by Castella et al. (64). These authors used a protected specimen brush, such as that designed by Wimberly et al. (35), in a heterogenous group of 38 patients. The brush was extracted from the catheter, and in the proximal extreme an intramuscular needle was inserted, through which two aliquots of 20 ml of saline solution were introduced. Three endoscopic tools were introduced in sequence: classic or conventional BAL, P-BAL and bronchial smear by means of a PSB. The sensitivity of P-BAL (95 %) was greater than that of the PSB (55 %), while specificity for both tools was similar (94 and 89 %, respectively). The specificity obtained using the classic BAL was far inferior to the values obtained with the other two techniques (42 %).

Recently, we described another variation of BAL, which we refer to as 'protected alveolar lavage' (PAL) (65). For this, we used a Combicath catheter with an external diameter of 1.7 mm, such as

Table 2: Methods used to perform protected bronchoalveolar lavage.

Reference (no.)	Year	No. of samples	FOB	Cut-off value used	Type of catheter	Volume (in ml)
Gaussorgues et al. (59)	1989	13	no	none	arterial	150
Pugin et al. (60)	1991	28	no	BI	single	100
Rouby et al. (61)	1993	69	no	none	protected double	20
Meduri et al. (63)	1991	46	yes	10^4 cfu/ml	protected balloon-tipped	150
Castella et al. (64)	1991	31	yes	10^4 cfu/ml	protected specimen brush	40
Sanchez Nieto et al. (65)	1993	21	yes	10^4 cfu/ml	protected double	40

FOB: fiberoptic bronchoscopy; BI: bacterial index.

Table 3: Bacteriological results obtained by protected specimen brush (PSB) and protected alveolar lavage (PAL) in 21 patients.

Patient no.	Results of culture (count)		Percent cells with ICO
	PSB specimen	PAL specimen	
1	negative	negative	0
2	Pseudomonas spp. (2×10^5)	Pseudomonas spp. (2×10^5)	65
3	negative	negative	0
4	negative	negative	0
5	negative	negative	0
6	Legionella pneumophila	Legionella pneumophila	0
7	negative	Staphylococcus aureus (2×10^4)	0
8	Klebsiella pneumoniae (1×10^4)	Klebsiella pneumoniae (5×10^5)	26
	Acinetobacter calcoaceticus (2×10^3)	Acinetobacter calcoaceticus (3×10^5)	
9	Pseudomonas aeruginosa (2×10^3)	Pseudomonas aeruginosa (4×10^5)	75
	Candida albicans (2×10^2)	Candida albicans (2×10^3)	
10	Staphylococcus aureus (5×10^2)	negative	0
11	negative	Candida albicans (1×10^3)	0
12	negative	Streptococcus pneumoniae (5×10^4)	5
13	negative	Staphylococcus aureus (2×10^4)	15
14	Acinetobacter calcoaceticus (1×10^4)	Acinetobacter calcoaceticus (1×10^5)	30
15	Acinetobacter calcoaceticus (2×10^3)	Acinetobacter calcoaceticus (1×10^5)	60
16	negative	negative	0
17	negative	negative	0
18	negative	negative	0
19	Acinetobacter calcoaceticus (2×10^3)	Acinetobacter calcoaceticus (1×10^5)	94
20	Legionella pneumophila	Legionella pneumophila	0
21	negative	herpes virus*	0

*Herpes virus identified by cytopathic changes.

that used by Rouby et al. (62), which we introduced through the work channel of a standard fiberoptic bronchoscope. Once wedged, the fiberoptic bronchoscope is placed into a subsegmented bronchi, in accordance with the radiographic location of the infiltrate and/or the direct observation of endobronchial secretions. Next, a Combicath catheter is introduced, and once the distal plug is expelled, the internal catheter is moved inside approximately 3 cm beyond the end of the external catheter. Eight 5 ml aliquots of isotonic sterile saline are then instilled, and suctioning is performed manually after every instillation. Fifty percent of the fluid obtained is processed for cytologic study and the other 50 % for bacteriologic study, including quantitative culture. We studied 20 patients suspected to have pneumonia and compared the bacteriological results obtained with the results of two consecutive uses of PSB and PAL, using a cut-off point of 10^3 cfu/ml and 10^4 cfu/ml, respectively. The proportion of positive results with the samples obtained by PAL was 57 % as opposed to 38 % with PSB. The proportion of cells containing intracellular organisms exceeded 7 % in 7 of the 12 positive cultures obtained with PAL. However, in two cases of Legionella pneumonia, no cells containing intracellular organisms were detected. Cytological examination of the sample obtained

by PAL resulted in the detection of one case of viral pneumonia, based on typical cytopathic inclusions. The bacteriological results obtained by both methods, PSB and PAL, are outlined in Table 3.

Recently, Rouby et al. (61) described meticulously the histologic and bacteriologic aspects of pneumonia in patients with mechanical ventilation. At the same time, they evaluated the diagnostic benefit of a protected mini-BAL technique carried out 'blindly', or nonbronchoscopically (non-B-mini-P-BAL), in a clinical model using a unilateral post-mortem lung test. Eighty-three critically ill patients on mechanical ventilation were studied. In 69 of these patients, non-B-mini-P-BAL was performed shortly before death. The good results obtained with this method, known as 'blind lavage', were attributed to two aspects. First, the catheters are introduced by means of an artificial airway without bronchoscopic help and descend spontaneously to the lower lung lobules. Second, the lesions occurring in nosocomial bronchopneumonia are usually distributed in the lower lobules. In ten patients with positive lung biopsy cultures and without evidence of alveolar infection, histologic evidence of bronchiolitis was found. Lesions were associated with bacterial concentrations of 10^5 cfu/g. These authors con-

cluded that a cut-off point of 10^4 cfu/g provides an inaccurate indication of the rate of active bronchopulmonary infection in patients receiving antibiotics. False-negative results obtained with the mini-BAL were not linked to pneumonia 'sterilised' by antibiotic treatment, and false-positive results often were not related to bronchiolitis. The sensitivity and specificity of the non-B–mini-P-BAL was 70 %.

Discussion

Until a few years ago, three tools had been widely used for the diagnosis of conventional bacterial pneumonia: analysis of sputum or endotracheal aspirates (43, 66–68), analysis of samples obtained by percutaneous ultrathin needle aspiration, perfected by Zavala and Schoell (69) and Dorca et al. (70), and examination of bronchial smears obtained by the PSB, described by Wimberly et al. (35, 36). The first method is inaccurate, with high sensitivity and low specificity (43, 66). In recent years a number of studies have evaluated the efficacy of quantitative bacterial culture in intubated patients with suspected pneumonia. An increase in the efficacy of this method was shown using a cut-off value of 10^6 cfu/ml (67) or 10^5 cfu/ml (68). Further studies are indicated in order to validate this diagnostic tool.

The second method offers excellent specificity, approaching 100 %, but is less sensitive than other invasive tools (71). In addition, most authors reject its use in mechanically ventilated patients due to the risk of pneumothorax. Only two studies of this method have been performed in humans on mechanical ventilation (66, 72). The last tool mentioned, examination of bronchial smears, also presents the problem of false-negative results, which, for some authors, renders it unacceptable, especially in critically ill patients (73, 74). These problems have stimulated research for alternative diagnostic tools such as BAL. Bronchoalveolar lavage achieves a greater and more representative volume of secretions from the lower respiratory tract than the other tools mentioned. Its role in the detection of opportunistic and obligated pathogens in immunosuppressed patients has been substantially established, but its value in the rapid identification and diagnosis of bacterial pneumonia is still under investigation. Figure 1 shows the main diagnostic indications for the variations of BAL in patients suspected to have pneumonia.
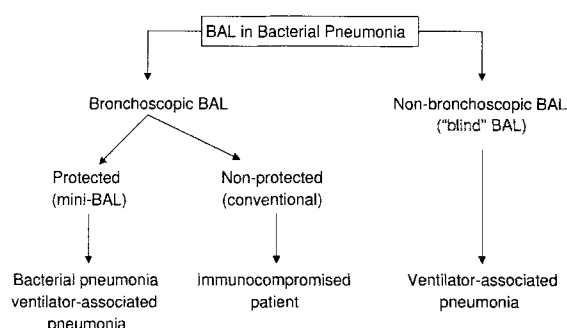
Figure 1: Indications for variations of bronchoalveolar lavage in the diagnosis of bacterial pneumonia.

The employment of culture quantification techniques has improved the specificity of the BAL in the diagnosis of bacterial lung infection (23, 33). Nevertheless, it is difficult to reach a consensus on when to fix a cut-off point that establishes a safe diagnosis of infection, above all in patients receiving antibiotics. In these patients a decrease in the bacterial count relative to the pre-established cut-off point may indicate either an absence of pneumonia or a diminishing of the bacterial charge due to the administered antibiotic. On the other hand, an increase may represent active pneumonia due to resistant bacteria or colonisation of the lower airway without lung tissue infection.

The difference in the cut-off point of bacterial growth, established by various authors as 10^3 to 10^5 cfu/ml, depends mainly on the type of bacteriologic processing and on three variables (32, 34, 52, 54): the concentration of pathogenic microorganisms in the lung area reached by BAL, the dilution attained with the lavage liquid and the final amount recovered (24). The dilution of lung secretions in the BAL fluid varies from 10 to 100 times; therefore, a count of 10^4 colonies is equivalent to 10^5 or 10^6 bacteria per millilitre, the figure considered indicative of lung infection (75, 76). However, in the presence of antibiotic treatment, it is impossible to attain 100 % sensitivity with BAL and other invasive tools based on the identification of bacteria in distal respiratory samples, since some lungs with histologic indications of pneumonia in patients receiving antibiotic treatment are 'sterile' (61). In patients without mechanical ventilation, the manner of administering the topic anaesthesia also influences the sensitivity of BAL. On one hand, the anaesthetic inoculates the bronchial tree against the microorganisms that accumulate in the channel of the fiberoptic bronchoscope, and on the other hand, lidocaine has bacteriostatic properties. However,

the concentrations of the anaesthetic upon dilution by the BAL fluid do not seem sufficient to inhibit bacterial growth (34, 77, 78).

The specificity of BAL in excluding bacterial pneumonia depends on whether colonising bacteria of the oropharynx and tracheobronchial tree are recovered. Despite measures taken to diminish contamination of the interior of the fiberoptic bronchoscope channel, most authors are inclined to look for technical solutions that minimise the risk of contamination of the BAL fluid. The protected BAL was designed to increase specificity over that of conventional BAL through the use of special catheters that impede contact between colonising bacteria and the instilled liquid (62–65). In our work the term 'protected alveolar lavage' (65) seems most adequate to differentiate protected lavage from classic or non-protected BAL. The introduction of catheters distally, to the end of the fiberoptic bronchoscope, washes or clearly shows the small airway and the alveolus. It is clear that the first results obtained using the various types of protected lavages improved the specificity over that of other methods. Until recently, the use of the PSB was considered optimal in mechanically ventilated patients with bacterial pneumonia (64). The use of PAL without bronchoscopic help seems a valid alternative. Besides being less expensive, PAL is a simple method carrying a lower risk of iatrogenic repercussions in patients on mechanical ventilation (60–62). Studies conducted thus far have described the diagnostic benefits made possible by protected lavage when used with an endoscopic guide. The diagnostic benefit obtained by applying rapid methods enabling direct visualisation of intracellular organisms in the BAL fluid is still controversial. The identification and quantification of 'infected cells' put forward by Chastre et al. (52) raises questions about sensitivity. The visualisation of various intracellular microorganisms, such as Legionella, may be difficult, with habitual staining occurring (65). The cut-off point suggested by these same authors, between 5 and 7 %, has not been compared with the results of others studies, and it has recently been stated that "antibiotic treatment can reduce significantly the presence of infected cells" in the BAL and thus affect the predictive value of subsequent cultures of PSB specimens (79).

The cytologic processing of BAL fluid, used systematically, does not seem to offer diagnostic advantages. Different studies have shown an increase in the percentage of neutrophils in patients with active pneumonia (59, 60, 65), but these neutrophils are not diagnostic and can be associated with pulmonary processes that are inflammatory but non-infectious (80). In those patients with pneumonia suspected to be caused by a virus or opportunistic microorganisms or presumed to be due to non-infectious pathology such as alveolar haemorrhage, it is suggested that a cytologic test with special staining be performed (63–65).

Other research tests, such as detection of pneumonia markers, have been described, including the visualisation of elastin fibres in tracheal aspirates (81), applied more recently to BAL analysis (60). Although the presence of elastin fibres is very specific in the existence of necrotising bacterial pneumonia, sensitivity is low and false-positive results may occur in patients with adult respiratory distress syndrome (82). Figure 2 summarises the main applications of BAL and PAL for the diagnosis of pneumonia.

Most studies have reported rates of false-negative results of 30 to 40 % with BAL (83, 84). Several factors account for this. First, there is the lack of standardisation of sampling techniques used by different investigators. Second, the sensitivity of BAL will be reduced if the patient is receiving antibiotics at the time of sampling. It is possible that the frequency with which false-negative findings occur depends not so much on the sampling technique as on whether and in how many doses appropriate antibiotics were administered prior to specimen collection and whether the pathogens concerned are sensitive to ongoing therapy. Finally, false-negative results may also occur with cultures containing bacterial counts at the cut-off value (10^4 cfu/ml), since some of the cultures originally judged negative may have represented early infection (85).
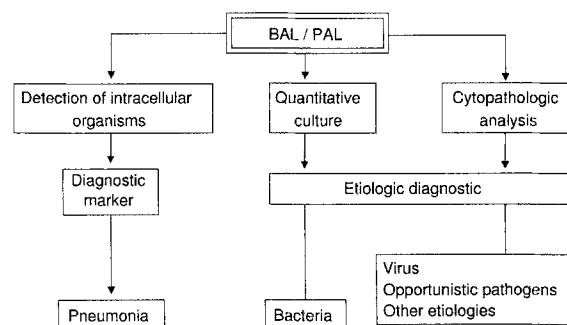
Figure 2: Applications of BAL and protected alveolar lavage (PAL) for the diagnosis of pneumonia.

Likewise, false-positive results are caused by a number of factors (83, 84). First, upper airway organisms can contaminate the BAL fluid during the procedure. The P-BAL was designed to avoid the contamination of this fluid. Second, prior antibiotic therapy can lead to overgrowth of antibiotic-resistant organisms, causing a false-positive result (86). Finally, false-positive results can occur in the setting of heavy colonisation of the airway. In one recent study in ventilated patients without pneumonia, Rodriguez de Castro et al. (87) found a significant relationship between the ability of the PSB and/or BAL to recover microorganisms and the duration of mechanical ventilation.

Bronchoalveolar lavage is a tool that presents very few complications. In fact, most of the undesirable effects are related to the use of fiberoptic bronchoscopy. It is rare that severe complications, such as pneumothorax, arise (88). Hypoxemia and arrhythmias can occur during use of the fiberoptic bronchoscope, but this is usually foreseen by an increase in the FIO_2 (89–92). Arterial hypoxemia is more pronounced and lasting in patients with pneumonia than in those without lung infection, possibly due to the alveoli being filled by the instilled liquid (93). Recently, it has been proposed that BAL procedures, bronchoscopic or other, could be responsible for a translocation of endotoxins from the infected or colonised airways to the interior of the lung capillaries or lymphatic channels through an alveoli capillary wall damaged by the infection. This passage of endotoxins or bacterial products in circulation could be responsible for a systemic sepsis-like response, with fever and hypertension after the use of BAL (93). The type and severity of these changes have yet to be clarified. The use of variations of BAL requiring a smaller amount of saline solution and/or the use of lavages without bronchoscopy should diminish the frequency and intensity of these adverse effects, although these hypotheses must still be confirmed.

Conclusions

Important advances in the use of BAL in the diagnosis of bacterial pneumonia have been made, but important questions also have yet to be answered. The first international conference on the clinical investigation of pneumonia associated with mechanical ventilation (26) recommended the use of the quantitative cultures of BAL specimens and established a cut-off point of 10^4 cfu/ml.

It was also recognised that contradictory results will continue to be reported, especially when treatments such as antibiotics and prolonged mechanical ventilation co-exist. The mini-protected BAL and the non-bronchoscopic BAL are other important diagnostic options, above all in pneumonia linked to mechanical ventilation. As these methods become further refined, they may even replace the PSB or conventional BAL, methods previously considered state of the art. It remains to be seen which of the variations of protected lavage will become of greatest diagnostic value and under which clinical circumstances.

It is hoped that there will be a prompt standardisation of the type of material used and of the technical procedures to be followed. It is still not known whether the systemic application of these and other invasive tools improve the prognosis of patients with nosocomial pneumonia. Although the use of BAL in the diagnosis of bacterial pneumonia in immunocompromised patients is still under research, the introduction of new variations such as the mini-protected-BAL has contributed to an increasing number of indications for bronchoalveolar lavage.

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