Diagnostic Value of Protected BAL in Diagnosing Pulmonary Infections in Immunocompromised Patients*

Rosa Jolis, PhD; Joan Castella, PhD, FCCP; Carmen Puzo, PhD; Pere Coll, PhD; and Carmen Abeledo, MD

Study objectives: To assess the diagnostic utility of protected BAL (P-BAL) in respiratory infections in immunocompromised patients and to examine whether P-BAL alone could substitute the combined use of protected specimen brush (PSB) and BAL in such patients.

Patients and study design: Thirty-seven immunocompromised patients who underwent PSB, P-BAL, and BAL simultaneously for the diagnosis or exclusion of bacterial or nonbacterial opportunistic respiratory infections were studied prospectively. The P-BAL was performed through the inner catheter of a telescoping plugged catheter with 60 mL of saline solution.

Main results: Thirteen (35%) cases of bacterial pneumonia were diagnosed. PSB obtained seven true-positive (TP) results, P-BAL obtained nine, and BAL obtained eight TP. Results of the three techniques were positive and concordant in 6 of the 13 cases. PSB remained free of contamination from oropharyngeal flora in all cases, P-BAL was contaminated twice, and BAL was contaminated in four cases. Opportunistic respiratory infections were diagnosed in 19 patients. P-BAL results were identical to those with BAL in all cases: 18 TP and 1 false-negative. The average volume of P-BAL fluid retrieved was 19 mL, sufficient for all microbiologic and cytologic processing. P-BAL was more time-consuming than both PSB and BAL procedures and was technically more complex.

Conclusion: P-BAL alone can substitute the combined use of both PSB and BAL in immunocompromised patients and attains a higher sensitivity than PSB in diagnosing bacterial pneumonia. The combined strategy continues to be a good choice, but due to the high incidence of bacterial pneumonia in these patients, a highly efficient diagnostic procedure is required not only for nonbacterial opportunistic respiratory infections but also for bacterial pneumonia.

(CHEST 1996; 109:601-07)

Key words: diagnosis; immunocompromised patients; pneumonia; protected bronchoalveolar lavage; respiratory infections

Pneumonia is the most common life-threatening infection in immunocompromised patients, causing 40% of deaths in this population.1 Etiologic agents include Pneumocystis carinii, cytomegalovirus (CMV), various fungi, Mycobacterium tuberculosis, atypical mycobacteria, and bacteria. According to different authors,2,3 bacteria can cause from 10 to 23% of pneumonia in AIDS patients. Although respiratory infections are common causes of pulmonary infiltrates in these patients, they must be distinguished from other conditions such as occult hemorrhage, malignant disease, pulmonary drug reactions, and nonspecific interstitial pneumonitis.1 Invasive procedures, especially lung biopsy, are often needed in these cases to reach a specific diagnosis.4 Regarding respiratory infections, determination of the various etiologic agents usually requires an invasive diagnostic procedure, as clinical features and radiographic findings are nonspecific, and noninvasive techniques are frequently non-diagnostic.

Different studies5-8 have demonstrated the utility of
the protected specimen brush (PSB) in diagnosing bacterial pneumonia in both nonintubated and mechanically ventilated patients. Furthermore, the diagnostic yield of BAL in the assessment of pulmonary infiltrates in immunocompromised patients has been widely described.\(^9\text{--}^{12}\) Consequently, and also because of the high incidence of bacterial pneumonia in immunocompromised patients, combined PSB and BAL has been suggested as the choice approach in the evaluation of these patients.\(^3\text{,}^{13}\text{,}^{14}\)

A new diagnostic technique for bacterial pneumonia has recently been described, especially for mechanically ventilated patients: protected BAL (P-BAL). This procedure has achieved good results with a higher sensitivity than PSB\(^15\text{--}^{17}\) and a better specificity than BAL.\(^18\) To our knowledge, however, the usefulness of P-BAL in diagnosing nonbacterial opportunistic infections has not yet been evaluated.

The aim of this study was to assess the P-BAL diagnostic value in opportunistic respiratory infections and to compare this with BAL and PSB to find out if a P-BAL alone could substitute the combined use of a PSB and a BAL in these patients. Theoretically, a higher sensitivity than PSB in diagnosing bacterial pneumonia and a better tolerance than PSB plus BAL could be obtained with P-BAL.

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**Table 1—Causes of Immunosuppression in All Patients (n=37)**

<table>
<thead>
<tr>
<th>Causes</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>23</td>
</tr>
<tr>
<td>Cardiac transplant recipient</td>
<td>6</td>
</tr>
<tr>
<td>Renal transplant recipient</td>
<td>3</td>
</tr>
<tr>
<td>Leukemia</td>
<td>2</td>
</tr>
<tr>
<td>Hodgkin's lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Bone marrow transplant</td>
<td>1</td>
</tr>
<tr>
<td>Weber-Christian panniculitis</td>
<td>1</td>
</tr>
</tbody>
</table>

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**Materials and Methods**

**Patients**

Over a 1-year period (1993), we prospectively studied 37 immunocompromised patients (19 men, 18 women; mean age, 38.5 years; range, 24 to 75 years) with pulmonary infiltrates and fever. Patients included in the study were cardiac, renal, and bone marrow transplant recipients, patients with disorders associated with altered immune function (AIDS, hematologic malignancy), and patients requiring treatment with immunosuppressive agents or corticosteroids (Table 1). In 2 of the 37 cases, patients were receiving mechanical ventilation. Infiltrates involved one lobe in 18 patients (48.6%) and were diffuse in 19 (51.4%). No pleural effusion was detected in any case. In nine cases, patients had been receiving antibacterial treatment for at least 48 h before the bronchoscopy and six subjects had already begun an anti-Pneumocystis therapy when fiberoptic bronchoscopy was performed.

**Protocol**

In the two mechanically ventilated patients, the fiberoptic bronchoscope (BF-P2OD or BF-P10; Olympus, Barcelona, Spain) was introduced through the endotracheal tube using a special adaptor after adequate sedation with short-lived paralysis. The ventilator setting was adapted appropriately during the procedure to improve ventilation and oxygenation, and the bronchoscope was introduced without bronchial aspiration. In nonmechanically ventilated patients, a minimum amount of local anesthesia (2% lidocaine solution) was administered, always with the patient in supine position, and the bronchoscope was then introduced nasally without bronchial aspiration. In the same order to avoid bacterial contamination from the bronchoscope channel, PSB, P-BAL, and BAL samples were then retrieved from three neighboring peripheral bronchi. Samples were taken from the lobe with maximal involvement according to the radiography or from the middle lobe or lingula in patients with diffuse pneumonia.

PSB was performed following a standardized technique described elsewhere\(^5\text{,}^{10}\) and aseptically cut into a sterile tube containing 1 mL of Ringer's lactate. To perform P-BAL, a new telescoping plugged catheter was introduced into the suction channel of the bronchoscope. The external catheter was protruded through the bottom of the bronchoscope and the distal plug was expelled by the brush and was then removed. The bronchoscope was then wedged in the selected peripheral bronchus and the inner catheter protruded. After screwing a perfusion needle into its proximal orifice to connect a syringe (Fig 1), P-BAL was carried out.

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**Figure 1.** P-BAL technique. The perfusion needle screwed into the proximal orifice of the catheter to connect the syringe.
by sequentially instilling and aspirating three 20-mL aliquots of sterile saline solution. Finally, BAL was performed with 100 mL of sterile saline solution infused through the working channel of the bronchoscope and then withdrawn by hand suction into the syringes used for infusion. All samples were transported to the laboratories within 15 min. In all cases, blood cultures were done before any protocol procedure.

Microbiologic Processing

The tube containing PSB was vortexed for 1 min to suspend all material from the brush, and BAL and P-BAL fluids were homogenized using repeated aspirations with a Pasteur pipette. Serial dilutions of each sample (1/10, 1/100, and 1/1,000) were prepared in saline solution; 0.1 mL of undiluted sample and each sample dilution were inoculated into the following culture agar media: 5% sheep blood, chocolate, eosine methylene blue (EMB), Wilkins-Chalgren anaerobe agar, buffered charcoal yeast extract (α-BCYE), and Sabouraud dextrose. Plates were evaluated for growth at 24 and 48 h and discarded after 5 days except for EMB and α-BCYE, which were evaluated at 7 days, and Sabouraud, which was evaluated at 4 weeks. BAL and P-BAL were also decontaminated by the NaOH-N-acetyl cysteine method and inoculated in Lowenstein-Jensen tubes and vials (Bactec 12A; Becton-Dickinson; Sparks, Md) that were incubated for 5 weeks before being discarded as negative. Except for Legionella pneumophila and mycobacteria, bacterial count of 10⁶ cfu/mL or more was the cutoff point to diagnose pulmonary infection by PSB and 10⁸ cfu/mL or more by P-BAL and BAL. Gram’s and Ziehl-Neelsen stains and Grocott methenamine silver for P carinii were performed using undiluted samples. Viral cultures were done in roller tubes. Cell cultures used were MRC-5, A 549, and Hep-2. The shell-vial assay was used for detection of CMV early antigen. All microorganisms isolated were identified by standard laboratory methods.²⁰ The expression “normal flora” was used to indicate contamination of BAL with a mixed flora of oropharyngeal bacteria.

The diagnosis of bacterial pneumonia was made when, following clinical and radiologic suspicion of respiratory infection, at least one of the following criteria was present: (1) a positive blood sample culture obtained 48 h before or after performing bronchoscopy; (2) a good response to an antibiotic treatment with absence of an alternative diagnosis.

RESULTS

Bacterial Pneumonia

Of the 13 (35%) cases of bacterial pneumonia, 10 were diagnosed by a positive respiratory sample. In six of the ten cases, results of the three techniques were positive and concordant. PSB and P-BAL, P-BAL alone, and both BAL alone were positive in one patient for each case. The other three cases of bacterial pneumonia were diagnosed by a positive blood culture in one case, and by clinical and evolutive criteria in two cases.

PSB did not obtain any contamination from oropharyngeal flora, P-BAL was contaminated twice (in one patient with nonbacterial pneumonia and in another without pulmonary infection), and BAL in four cases (in one case the causative agent was also isolated by PSB, and results in one patient were interpreted as a false-negative and a false-positive) (Table 2).

In 8 of the 37 patients (21%), bacterial pneumonia was the only cause of pulmonary infiltrates and 5 patients (13.5%) were diagnosed from both bacterial and nonbacterial opportunistic infections. Finally, in 24 patients, a diagnosis of bacterial respiratory infection was ruled out since all the samples were negative and there was a clinical and radiologic improvement without antibacterial treatment. The most commonly isolated microorganism was Streptococcus pneumoniae (6 of 11 cases). Microorganisms isolated from all the samples in significant concentrations are shown in Table 3.

Opportunistic Microorganisms Pneumonia

BAL analysis revealed the diagnosis of 18 cases of opportunistic pneumonia. P-BAL obtained identical results to BAL in all cases. P carinii was detected in ten cases, all patients with AIDS; viruses in ten cases (six CMV, two herpes simplex virus, one respiratory syncytial virus, and one influenza A virus); fungi in two cases (one Cryptococcus neoformans and one Scopulariopsis sp) and, finally, mycobacteria in three cases (two M tuberculosis and one Mycobacterium avium-intracellulare). Both BAL obtained one false-negative result in a patient who was diagnosed as having M tuberculosis infection by a positive Lowenstein-Jensen culture of sputum. This patient had received antiy-cobacterial drugs for 4 days before bronchoscopy was performed. Diagnosis of opportunistic infection was rejected in 18 patients due to the negativity of all the samples and the clinical evolution. In 6 of 10 patients who were diagnosed as having P carinii pneumonia, an

Table 2—Diagnostic Efficiency of the Three Techniques in Both Kinds of Pulmonary Infection

<table>
<thead>
<tr>
<th></th>
<th>Bacterial</th>
<th></th>
<th>Nonbacterial</th>
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<tbody>
<tr>
<td></td>
<td>PSB</td>
<td>P-BAL</td>
<td>BAL</td>
<td>P-BAL</td>
</tr>
<tr>
<td>True-positives</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>False-positives</td>
<td>0</td>
<td>2</td>
<td>4*</td>
<td>—</td>
</tr>
<tr>
<td>True-negatives</td>
<td>24</td>
<td>22</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>False-negatives</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>53</td>
<td>69</td>
<td>61</td>
<td>95</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>100</td>
<td>91</td>
<td>84</td>
<td>—</td>
</tr>
</tbody>
</table>

*In one case, the causative agent was also isolated (a true-positive plus a false-positive) and in another, the result was a false-negative and a false-positive at the same time.
anti-"P. carinii" treatment had been administered for 5
days previously in 1 case, for 3 and 2 days in 2 cases,
and for 24 h in the rest. According to these results, the
overall diagnostic yield of both BAL in diagnosing op-
portunistic infections was 95% (18 of 19 cases) (Table
2). Opportunistic microorganisms isolated from all the
samples and the kind of immunosuppression in each
patient are presented in Table 3.

<table>
<thead>
<tr>
<th>Case</th>
<th>Background</th>
<th>Ant</th>
<th>PSB</th>
<th>P-BAL</th>
<th>BAL</th>
<th>Blood</th>
<th>Others</th>
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<tbody>
<tr>
<td>1</td>
<td>Renal T</td>
<td>No</td>
<td>—</td>
<td>CMV</td>
<td>CMV</td>
<td>CMV</td>
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<tr>
<td>2</td>
<td>Bone marrow transplant</td>
<td>No</td>
<td>—</td>
<td>H influenzae</td>
<td>H influenzae</td>
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<td>3</td>
<td>Leukemia</td>
<td>No</td>
<td>S pneumoniae</td>
<td>S pneumoniae</td>
<td>S pneumoniae</td>
<td>—</td>
<td>—</td>
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<tr>
<td>4</td>
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<td>Yes</td>
<td>C neoformans</td>
<td>C neoformans</td>
<td>P carinii</td>
<td>P carinii</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Renal T AIDS</td>
<td>Yes</td>
<td>—</td>
<td>S pneumoniae</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Leukemia</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>7</td>
<td>AIDS</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>S pneumoniae</td>
</tr>
<tr>
<td>8</td>
<td>Cardiac T</td>
<td>No</td>
<td>CMV RSV</td>
<td>CMV RSV</td>
<td>CMV</td>
<td></td>
<td>Urine, CMV pos</td>
</tr>
<tr>
<td>9</td>
<td>AIDS</td>
<td>Yes</td>
<td>S pneumoniae</td>
<td>S pneumoniae</td>
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<tr>
<td>10</td>
<td>AIDS</td>
<td>Yes</td>
<td>S pneumoniae</td>
<td>M tuberculosis</td>
<td>M tuberculosis</td>
<td>M tuberculosis positive</td>
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<tr>
<td>11</td>
<td>Panniculitis Weber-Christian</td>
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<td>Scopulariopsis</td>
<td>Escherichia coli</td>
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<td>13</td>
<td>AIDS</td>
<td>Yes</td>
<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>14</td>
<td>Renal T</td>
<td>Yes</td>
<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>AIDS</td>
<td>Yes</td>
<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>AIDS</td>
<td>Yes</td>
<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>AIDS</td>
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<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>AIDS</td>
<td>No</td>
<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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<tr>
<td>19</td>
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<td>P carinii</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>20</td>
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<td>No</td>
<td>M tuberculosis</td>
<td>M tuberculosis</td>
<td>M tuberculosis positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>AIDS</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*Anti=antibiotic treatment; Renal T=renal transplant recipient; Cardiac T=cardiac transplant recipient; Pc=anti-"P. carinii" treatment; TBC=antimycobacterial drugs; CSF=cerebrospinal fluid. HSV=herpes simplex virus; RSV=respiratory syncytial virus; Pos=positive.

1Mechanically ventilated.

1MRSA=methicillin-resistant "Staphylococcus aureus."
Course of Pulmonary Disease

Owing to the results obtained by the three techniques, treatment was modified in 14 cases (52%). New therapy was initiated in six cases and empiric therapy was discontinued, replaced, or completed in eight. Clinical and chest radiographic improvement was observed in 24 of the 27 episodes of pulmonary infection (89%). The mortality was 11% as a result of pulmonary infection.

P-BAL Characteristics

The average volume of P-BAL fluid recovered was 19 mL (range, 8 to 40 mL), sufficient in most cases to perform all microbiologic processing. Although bronchoscopy was well tolerated in most cases and no severe complications occurred, more time was spent in performing P-BAL than PSB and BAL procedures (P-BAL: 180±18; PSB and BAL: 120±11 s). Besides, P-BAL is technically more complex since correct practice can be difficult if vision of the bronchial tree is poor, as occurs when there is a considerable amount of respiratory secretions.

Cost Analysis

Because the three techniques are performed through a fiberoptic bronchoscope, their cost has been evaluated taking into account only the material and cultures needed to perform a simple P-BAL, on one hand, and a PSB and a BAL, on the other. The cost of the material used for P-BAL (syringes, plastic recipients, telescoping plugged catheter) is therefore about $31, and approximately $32 for PSB and BAL (Ringer’s lactate tube, telescoping plugged catheter, syringes, plastic recipients). Cultures for bacteria, mycobacteria, viruses, parasites, and fungi are necessary for both techniques at a cost of about $495. According to this analysis, it can be concluded that the costs of the two strategies are very similar.

Discussion

Several years ago, transbronchial lung biopsy was the main technique for diagnosing respiratory infections in immunocompromised patients, but, since the AIDS era, different authors have demonstrated the high diagnostic yield of BAL in opportunistic infections. Its sensitivity varies according to the different kinds of immunosuppression. In AIDS patients, its diagnostic yield is 94 to 98%. Despite this, Jules-Elysee and coworkers and Salzman and Rosen recently found a lower sensitivity for BAL in diagnosing P. carinii infection in AIDS patients who had received a prophylactic treatment against this organism. These authors therefore recommend performing a transbronchial lung biopsy in such patients. Further investigation is needed to confirm these findings.

However, a high incidence of bacterial respiratory infections in immunocompromised patients has been reported and, because BAL can be contaminated more easily from oropharyngeal flora than PSB, some authors have recommended the combined practice of these two procedures to simultaneously diagnose both kinds of pulmonary infections. Despite the good results with this strategy, it would be useful to have a single diagnostic procedure that could permit the diagnosis of both respiratory infections. Obtaining a greater sensitivity than PSB in the diagnosis of bacterial pneumonia would be an advantage. Different studies have shown good diagnostic efficiency of P-BAL in bacterial pneumonia, but its usefulness has not yet been demonstrated in nonbacterial opportunistic infections.

In our study, 13 cases of bacterial pneumonia (35%) were diagnosed and P-BAL obtained a sensitivity of 69% in diagnosing this kind of pneumonia, which was higher than PSB (53%) and similar to BAL (61%). These sensitivities are lower than those usually described for these procedures, but the limited number of patients with bacterial pneumonia and also the fact that nine had received previous antibiotic treatment should be taken into account. All three techniques obtained high specificity (PSB, 100%; P-BAL, 91%; BAL, 84%). The high specificity of BAL can be explained by the fact that some preventive measures to avoid sample contamination (such as aspiration of respiratory secretions before performing the procedures and a perfect wedge of the bronchoscope) were taken. Furthermore, in nonmechanically ventilated patients, the amount of respiratory secretions is not usually very significant and the risk of contamination is thus lower. These results suggest that P-BAL is a good procedure for diagnosing bacterial pneumonia, offering a greater sensitivity than PSB.

Kelly and coworkers found that a volume of at least 120 mL was necessary to withdraw secretions from peripheral lung fields when BAL procedures were performed. However, studies about the dilution effect on culture results are not available. Finally, in the International Consensus Conference about Clinical Investigation of Ventilator-Associated Pneumonia, a volume of saline solution of at least 140 mL to perform BAL procedures was recommended. Accordingly, it could be thought that the volume used in this study (60 mL) was not sufficient to withdraw alveolar fluid. However, P-BAL retrieved the same opportunistic organisms as BAL in most cases. This suggests that P-BAL can attain as good a diagnostic yield as BAL (95%) in nonbacterial opportunistic infections. However, the
average volume retrieved by P-BAL (19 mL) was sufficient for both microbiologic and cytologic processings in most cases. Thus, according to the results and the cost of the two strategies, it can be concluded that P-BAL alone could substitute the combined use of both PSB and BAL in diagnosing respiratory infections in immunocompromised patients. This is specially true when a high risk of contamination by BAL can be foreseen due to the amount of respiratory secretion in the bronchial tree or in patients with contraindications for PSB and BAL practice such as severe hemostatic disturbances or hypoxia. Despite this, we are of the opinion that the combined strategy continues to be a correct choice and the practice of a BAL alone could be an acceptable option in patients with poor tolerance and with few respiratory secretions if some preventive measures are taken.

Respiratory infections in immunocompromised patients can be caused by many kinds of organisms. Some are more frequently isolated than others, depending on the cause of the immunosuppression. The most common microorganisms in our study were *S. pneumoniae* in bacterial pneumonias and *P. carinii* (ten cases), CMV (six cases), and mycobacteria (*three M. tuberculosis* and one *M. avium-intracellulare*) in nonbacterial opportunistic infections. These results can be explained by the fact that 23 of the 37 patients had AIDS and these are the most common organisms in such patients as has been widely reported. In six of the ten patients diagnosed as having *P. carinii* pneumonia, appropriate treatment had been started before the procedures were performed. Although this number is too low for conclusions to be drawn, the fact that *P. carinii* was diagnosed in all cases supports the results obtained by Tu and coworkers. These authors suggest that empirical treatment can be started when there is reasonable suspicion of *P. carinii* pneumonia and that a bronchoscopy should be carried out only in patients not responding to 5 days of empiric therapy.

**CONCLUSION**

Our results show that P-BAL alone could substitute for the combined use of both PSB and BAL in immunocompromised patients, especially in AIDS subjects, attaining a higher sensitivity than PSB in diagnosing bacterial pneumonia. It could be of special interest in patients with relative contraindications for the other bronchoscopic techniques. The combined use of PSB and BAL continues to be a good choice. However, due to the high incidence of bacterial pneumonia among these patients (35% according to our results), in our opinion, a highly efficient diagnostic procedure is required not only in nonbacterial opportunistic infections but also in bacterial pneumonia. Further studies are necessary to determine whether P-BAL obtains these same good results in non-AIDS patients and in diagnosing noninfectious pulmonary infiltrates.

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