Diagnostic accuracy of bronchoalveolar lavage samples in immunosuppressed patients with suspected pneumonia: Analysis of a protocol

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KEYWORDS
Bronchoalveolar lavage; Immunocompromised patients; Pneumonia; Diagnostic accuracy

Summary
Background: Fast and accurate etiologic diagnosis of pneumonia in immunocompromised patients is essential for a good outcome. Utility of bronchoalveolar lavage (BAL) samples has already been established, but studies about them are scarce and limited to few countries. We aimed to evaluate the accuracy of a diagnostic protocol, emphasizing on local epidemiology, rapidity, and yield of different techniques.

Methods: One year prospective study of 101 consecutive immunosuppressed patients admitted with suspected pneumonia to a university hospital. They all had bronchoscopic BAL (n = 109) and respiratory sampling. Conventional microbiological studies, cytomegalovirus pp65 antigenemia and transbronchial biopsy (TBB), whenever considered pertinent,

Abbreviations: BAL, bronchoalveolar lavage; HUSVP, Hospital Universitario San Vicente de Paúl; TBB, transbronchial biopsy; FB, flexible bronchoscopy; ZN, Ziehl-Neelsen; TBO, modified toluidine blue; CFU, colony forming units; CMV, Cytomegalovirus; UAIC, unspecific airways inflammatory condition; PPV, positive predictive value; NPV, negative predictive value; TB, tuberculosis; PCR, polymerase chain reaction; CSF, cerebrospinal fluid

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Introduction

Pneumonia is a major cause of morbidity and mortality in immunosuppressed patients.1–3 Rapid diagnosis and early treatment are necessary for a good outcome.1,4 However, accurate microbiological diagnosis is challenging as many infectious and noninfectious conditions have comparable clinical presentation.1,2,5–8

Flexible bronchoscopy (FB) with bronchoalveolar lavage (BAL) is simple, safe, fast and reliable. It has been extensively used as diagnostic procedure for assessing immunosuppressed hosts with pulmonary infiltrates.7,9–14 BAL sensitivity and specificity are comparable to other techniques.15 Ideally, a clinician should submit BAL samples to a single microbiology laboratory, results of histochemical stains should be available within hours of specimen submission, and a report summarizing all of the information obtained from analysis should be issued from that laboratory.9 Several groups have already demonstrated the clinical value of BAL protocols, particularly when samples are methodically studied.7,9,10,16–18 However, they are limited to few countries.

Local data in Latin America, specifically in Colombia, are scarce.19,20 This study was conducted to evaluate the role of systematic analysis of BAL samples in the etiologic assessment of immunosuppressed patients with pneumonia, in a region with different social, economical and epidemiological patterns. In order to optimize the process, and elaborate guidelines for empirical treatment in these cases, we emphasized on the protocol diagnostic accuracy, rapidity to preliminary diagnosis, specific problems with some pathogens, and differences with other countries reports.

Materials and methods

Patient population

We included 101 immunosuppressed patients consecutively admitted to Hospital Universitario San Vicente de Paúl (HUSVP, Medellin-Colombia) with suspected pneumonia from June 2000 to July 2001. Patients older than 12 years were included if they had at least one of the following symptoms: cough, dyspnea, abnormal auscultatory findings and new pulmonary infiltrates. Immunosuppression was considered if: HIV infection or AIDS; neutropenia < 500 cells/mm³, bone marrow or solid organ transplantation, hematological malignancies, immunosuppressive treatment (prednisone 0.3 mg/k/d, or its equivalent, longer than 2 weeks), noncontrolled diabetes and splenectomy. The research team had no control over treatment. All patients signed an informed consent according to legal requirements (Colombia Ministry of Health Resolution 008430, 1993), and the study was approved by University of Antioquia Ethics Committee and HUSVP Internal Review Board. Exclusion criteria were pregnancy, severe heart arrhythmia, serious hemoptysis, instable hemodynamic status, critical respiratory failure (PaO₂/FiO₂ < 120) and any other condition with risk for bronchoscopic procedures.

Bronchoalveolar lavage and respiratory sampling

FB and BAL were performed following American Thoracic Society guidelines.21 Transbronchial biopsy (TBB) was done in case of diffuse interstitial or reticulonodular infiltrates (at pulmonologist’s discretion), whenever the patient had no contraindications for it (platelet count ≤ 50,000/mm³, increased coagulation times, significant pulmonary hypertension, or poor functional respiratory reserve). An open-lung biopsy was carried out if BAL studies were no diagnostic and clinical conditions allowed it.

Laboratory processing of specimens

Each specimen was quantitatively cultured for bacteria on conventional agar media. All isolates containing ≥ 10⁵ colony forming units (CFU)/ml were identified to specie. In general, BAL specimens were considered significant if they contained ≥ 10⁶ CFU and ≤ 1% squamous epithelial cells in Wright-stained slides.9,22–24 After qualitative cultures were set up, specimens were centrifuged at 1500g for 20 min at 4 °C. Pellets were partitioned. A portion was plated for fungus (incubated at 30 °C in aerobic conditions for 4 weeks...
on Mycosel, Girasol, and Sabouraud’s agar), and mycobacteria (at 37°C in aerobic conditions for 6 weeks on Ogawa-Kudoh medium and thin layer agar). The other portion was suspended in 4 ml of Hanks salt solution and cytocentrifuged at 1500 rpm for 15 min (Cytospin III, Shandon Instruments, Sewickley, PA) after adjusting to 10^5 cells/ml. Slides were stained with Wright, Gram, Ziehl-Neelsen (ZN), modified Kinyoun and modified toluidine blue (TBO), according to Kahn–Jones’ protocol.

Bronchial brushings, transbronchial and open lung biopsy specimens were stained with hematoxylin–eosin, ZN and Gomori-methenamine silver.

Variables recorded

Conventional clinical and laboratory data with special emphasis on type, stage and severity of immunosuppression, antibiotic’s use within previous week of respiratory sampling, and microbiological analysis of blood, sputum, urine, pleural and spinal fluids. Cytomegalovirus (CMV) pp65 antigenemia assay was evaluated in all cases.

All in-patients were followed until discharge or death. Survivors were followed up for 1 year through visits to the infectious diseases clinic and/or by reviewing their clinical charts. Telephone calls to patients, their families and doctors in charge were tried when they did not return to the hospital.

Diagnostic criteria

To determine whether an identified microorganism on BAL was a true pathogen, we applied strict diagnostic criteria (Table 1). Besides that, etiology was established based on other diagnostic procedures, clinical course and final outcome. At least two of the clinical investigators evaluated the information of every case. Unspecific airways inflammatory condition (UAIC) was defined by the presence of: (i) cough without dyspnea, (ii) unspecific auscultatory findings in absence of pulmonary infiltrates, (iii) spontaneous improvement without specific treatment, and (iv) no microorganisms isolated. Diagnoses of pulmonary fibrosis, malignancies, pulmonary hypertension or edema were based on clinical, radiographical and histopathological evaluation. If patients had pulmonary infiltrates, but histopathological and microbiological results were not conclusive, final diagnosis was classified as unclear.

Statistical analysis

Data were collected and analyzed using Epiinfo software, version 6.04 (Epidemiology Program Office, CDC, Atlanta, GA). Two-tailed Fisher’s exact test was used to compare pairs of proportions. A P-value <0.05 was considered statistically significant. Sensitivity, specificity, positive and negative predictive values (PPV–NPV), and accuracy (a+d/ a+b+c+d)\(^{27}\) were calculated using Epidat software, version 2.0 (Xunta de Galicia, Santiago de Compostela, España).

Table 1 Criteria to determine whether an identified infectious agent on BAL was a true pathogen

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Any positive stain and/or isolation of <em>P. jirovecii</em>, <em>M. tuberculosis</em> and <em>H. capsulatum</em>.</td>
<td></td>
</tr>
<tr>
<td>2 Isolation of any recognized bacterial pulmonary pathogen on a quantitative culture (\geq 10^5) CFU/ml and epithelial squamous cells (\leq 1%). Also, any bacterial growth was considered significant in patients who were on appropriate antibiotics for the isolated bacteria within the previous week, as long as the Gram stain was compatible and no other etiology was demonstrated. If culture was negative but the patient was on broad spectrum antibiotics when BAL was performed, bacteria were considered probable cause of pneumonia, as long as he/she improved with, and no other causes accounted for symptoms.</td>
<td></td>
</tr>
<tr>
<td>3 Isolation of <em>Cryptococcus neoformans</em> on BAL, when: (i) it was isolated concurrently in other sterile body fluid, such as blood or CSF; (ii) no other possible pulmonary pathogen was isolated; or (iii) there was tissue infiltration on histopathology.</td>
<td></td>
</tr>
<tr>
<td>4 Isolation of <em>Aspergillus</em> spp. in case of: (i) neutropenia or hematological malignancies; (ii) hemoptysis or alveolar hemorrhage; (iii) concurrent isolation in other sterile body fluid, such as pleural fluid; or (iv) tissue infiltration on pulmonary biopsy.</td>
<td></td>
</tr>
<tr>
<td>5 Isolation of any nontuberculous mycobacteria when there was no other pathogen accounting for the pneumonia.</td>
<td></td>
</tr>
<tr>
<td>6 Histopathological evidence of pulmonary damage due to invasive CMV or <em>Candida</em> spp.</td>
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</tr>
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</table>

BAL: bronchoalveolar lavage; CFU: colony forming units; CSF: cerebrospinal fluid; CMV: Cytomegalovirus.

Results

Patients

During the study, 109 BAL procedures were performed in 101 patients. Table 2 displays basal features of them. HIV infection was the cause of inclusion in 80 patients, 90% with AIDS. Those with two or more causes of immunosuppression were included in the most relevant category at time BAL was done.

Etiology of pulmonary symptoms

Table 3 shows the distribution of 122 final diagnoses. The most common etiologies were mycobacteria (26.7%), UAIC (23.8%, almost exclusively in AIDS patients), bacteria (18.8%) and *Pneumocystis jirovecii* (17.8%). Predominant etiology varied according to immunosuppression: *Mycobacterium* spp. (30%), UAIC (28.7%) and *P. jirovecii* (21.2%) in HIV/AIDS patients, aspergillosis (27.3%) in transplantation, and bacterial pneumonia (70%) in the others.
Diagnostic accuracy of the protocol

Infections accounted for 79/122 (64.8%) final diagnoses (Table 3). BAL samples allowed us to identify 97 agents, 60 of them considered true pathogens. Candida spp. and bacteria were common colonizers (Fig. 1). Only 3 of 43 noninfectious pulmonary pathologies were diagnosed by the protocol, all of them by TBB (Fig. 2). Thus, general yield was 51.6% (63/122). In seven cases, microorganisms initially identified on BAL as pathogens, were considered misclassified by further analysis. According to Table 1, sensitivity in pulmonary infections was 75.9% (IC95%: 64.8–84.6%), specificity 86.0% (72.6–93.7%), PPV 89.6% (79.1–95.3%), NPV 69.4% (56.2–80.1%) and accuracy 79.8% (71.7–86.2%). Sensitivity was 100% for P. jirovecii (18/18), histoplasmosis (6/6) and cryptococcosis (3/3), 70.4% for mycobacteria (19/27), 60% for aspergillosis (3/5) and 57.9% for conventional bacteria (11/19) (Fig. 2). Direct microscopy was positive in 36 of 49 patients (73.5%) with fungal and/or mycobacterial pneumonia (P. jirovecii 18/18, mycobacteria 10/19, histoplasmosis 3/6, aspergillosis 3/3 and cryptococcosis 2/3).

Mycobacteria were considered the cause of pneumonia in 27 patients, but this protocol only diagnosed 19/12 M. tuberculosis, 4 nontuberculous mycobacteria and 3 ZN-positive, but culture-negative cases. Of eight patients missed by the protocol, two had pulmonary tuberculosis (TB) and six had extrapulmonary forms (two mediastinitis, one pericarditis, one pleuritis, one lymphadenitis and one gastrointestinal TB). Three were already on tuberculosis treatment. Sensitivity of cultures and acid fast staining in BAL samples was 59% (16/27) and 37% (10/27), respectively; 13 of 16 positive cultures grew in thin layer, 13 in Ogawa and 10 in both media. Time required to growth was 22±10.7 days in Ogawa (range: 12–55; median: 20) and 14.3±6.0 in thin layer (range: 4–26; median: 15).

BAL samples allowed to detect 11/19 patients with bacterial pneumonia (57.9%). All cases not diagnosed by BAL corresponded to partially treated infections (mean: 6 days, range: 2–12), which improved with prescribed antibiotics.

Histopathological studies were performed in 28 patients: 24 TBB, 3 open lung biopsies and 1 necropsy. TBB identified 13 of 23 infectious agents (56.5%) demonstrated in these 24 patients. Sensitivity was 50% (5/10) for mycobacteria, 33% (2/6) for bacteria, 100% for P. jirovecii (3/3), and 75% for other fungal infections (3/4).

Diagnostic yield of other procedures

CMV disease was demonstrated in seven patients (six AIDS and one kidney transplant recipient). Of them, three had retinitis, three had colitis (by biopsy) and one, the transplantation case, had pneumonia associated to central nervous system involvement (by necropsy). Among these patients, antigenemia assay was negative in 4, low-positive in 2 (<10 positive cells/150,000 leukocytes) and highly positive (180/150,000) in 1 case (CMV ulcerative colitis). On the other hand, only 1 of 13 patients with pp65 ≥10 cells/150,000 leukocytes had CMV disease.

Chest X-rays had no significant value to predict the etiology of infection.

Discussion

Systematical study of BAL samples leads to accurate and opportune diagnosis of etiology in immunosuppressed patients with suspected pneumonia.1–4, 29 We found a general yield of 51.6%, but 75.9% in infectious diseases. These results are very similar to previous reports from
United States, Spain, whose general yield was 51–60%; for pulmonary infections was 52–81%, and the proportion due to infectious diseases 61–72% (65% in our study).

Distribution of pulmonary pathogens depends on the epidemiology of each region. In this study, *Mycobacterium* spp., mainly tuberculosis, affected one of every four immunosuppressed patients with pneumonia, unlike developed countries, where it involves 2–4% of them. It explained one-third of infections identified in this study (27/79, 34.2%).

### Table 3: Etiology of 122 final diagnoses in 109 BAL procedures made to 101 patients, according to type of immunosuppression.

<table>
<thead>
<tr>
<th>Final diagnosis, n (%)</th>
<th>HIV/AIDS (n = 80)</th>
<th>Transplant (n = 11)</th>
<th>Others* (n = 10)</th>
<th>Total (n = 101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterial infection**</td>
<td>24 (30.0)</td>
<td>2 (18.2)</td>
<td>1 (10.0)</td>
<td>27 (26.7)</td>
</tr>
<tr>
<td>UAIC†</td>
<td>23 (28.7)</td>
<td>1 (9.1)</td>
<td>0</td>
<td>24 (23.8)</td>
</tr>
<tr>
<td>Bacterial pneumonia</td>
<td>11 (13.7)</td>
<td>1 (9.1)</td>
<td>7 (70.0)</td>
<td>19 (18.8)</td>
</tr>
<tr>
<td><em>P. jiroveci</em></td>
<td>17 (21.2)</td>
<td>1 (9.1)</td>
<td>0</td>
<td>18 (17.8)</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>5 (6.2)</td>
<td>1 (9.1)</td>
<td>0</td>
<td>6 (5.9)</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>3 (3.7)</td>
<td>2 (18.2)</td>
<td>1 (10.0)</td>
<td>6 (5.9)</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>0</td>
<td>3 (27.3)</td>
<td>2 (20.0)</td>
<td>5 (4.9)</td>
</tr>
<tr>
<td>Unclear cause</td>
<td>2 (2.5)</td>
<td>2 (18.2)</td>
<td>0</td>
<td>4 (4.0)</td>
</tr>
<tr>
<td>Malignancy infiltration‡</td>
<td>1 (1.2)</td>
<td>0</td>
<td>3 (30.0)</td>
<td>4 (4.0)</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>2 (2.5)</td>
<td>2 (18.2)</td>
<td>0</td>
<td>4 (4.0)</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>3 (3.7)</td>
<td>0</td>
<td>0</td>
<td>3 (3.0)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>1 (1.2)</td>
<td>0</td>
<td>0</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>0</td>
<td>1 (9.1)</td>
<td>0</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Total diagnoses, ‡n</td>
<td>92</td>
<td>16</td>
<td>14</td>
<td>122</td>
</tr>
<tr>
<td>Infections, n (%)</td>
<td>60 (65.2)</td>
<td>9 (56.2)</td>
<td>10 (71.4)</td>
<td>79 (64.8)</td>
</tr>
</tbody>
</table>

*Others: hematomatous malignancies (6 patients), on therapy with corticosteroids (3), neutropenic patient (1).
**Mycobacterial infections: tuberculosis (19 patients), nontuberculous mycobacteria (4), culture negative cases (4).
†UAIC: unspecific airways inflammatory condition (see "Diagnostic criteria" in Materials and Methods).
‡Malignancy infiltration of lung: Kaposi sarcoma (1 case in a HIV/AIDS patient), anaplastic lymphoma (1), Hodgkin disease (1), multiple myeloma (1).

**Total diagnoses: the sum of each column is greater than the number of patients in each category as several patients had more than one diagnosis.

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**Figure 1** Distribution of the different germs isolated in BAL samples (n = 109) in 101 immunosuppressed patients with suspected pneumonia. Black bars correspond to microorganisms considered true pulmonary pathogens, and striped bars to colonizers, according to criteria displayed in Table 1. Numbers in front of bars represent the percentage of each isolated microorganism considered true pathogen.
50–84/100,000\(^{32}\) and all tropical Latin American region.\(^{32}\) Furthermore, the magnitude of the problem can be even worse because diagnosis is still elusive (the protocol missed 8/27 mycobacterial infections, 29.6%). There are several possible explanations for these false negative cases: (i) 3/8 were on TB treatment when BAL was carried out (on the 1st, 2nd and 5th month, respectively) and (ii) presence of interstitial more than alveolar involvement. Unfortunately, many clinicians do not use to culture pulmonary tissue; and (iii) we did not make TBB to everyone in the study. However, histopathological yield for mycobacteria was only 50%. Our results highlight the importance of other diagnostic tools, such as blood/tissue cultures and polymerase chain reaction (PCR) in BAL samples. Meanwhile, in high prevalence regions, clinicians should consider the empirical use of TB treatment, especially when no other pathogen is found responsible.

Frequency of histoplasmosis was similar to reports from USA in HIV patients,\(^{10}\) but greater than in Spain\(^{16}\) and non-HIV immunocompromised hosts.\(^{9,16,17}\) On the other hand, incidence of bacterial pneumonia (17%) and \(P.\ jiromei\) (16%) were within percentages reported elsewhere (7–27% and 2–47%); however, the occurrence of bacteria in our protocol (17%) could be underestimated by use of broad spectrum antibiotics before processing BAL samples.\(^{22,33}\) In fact, all eight patients with bacterial pneumonia, whose diagnosis was missed, were on antibiotics at the time of BAL (mean: 6 days).

Direct stains from most patients with fungal and mycobacterial infections were reported as positive within the first 6h after BAL (36/49, 73.5%). These preliminary results were timely enough to start early treatment. The faster growth of mycobacteria in thin layer agar than in Ogawa medium sped up 1 week identification and susceptibility testing of mycobacteria.

Noninfectious diseases explained most of missed diagnosis, as has also been reported elsewhere\(^7\) (Fig. 2). UAIC was the most common cause in our cases; it occurred in patients with decreased level of consciousness, whose impossibility to deal with bronchial secretions gives them cough and abnormal auscultatory findings. Most had opportunistic AIDS-related infections (such as CMV or toxoplasma encephalitis) and severe AIDS–dementia complex. They all got better after improving their respiratory hygiene and neurological conditions.

Our study had two important limitations. First, we did not include diagnostic assays to rule out the presence of “atypical” germs and/or respiratory viruses. According to previous studies,\(^9,16,17,34\) pneumonia could be caused by these agents in 17–19% of immunosuppressed patients. Nevertheless, several facts suggest it should not be a problem in our population. First, it is known that CMV is not a frequent cause of pneumonia in HIV/AIDS patients, who represented the majority of individuals involved (80/101).\(^{16}\) Secondly, \(Legionella\) spp. and \(Chlamydophila pneumoniae\) do not seem to be a big problem in South America, although information about them is limited\(^{35}\), and \(Mycoplasma pneumoniae\) is not especially common in these patients.\(^{16,17}\) Finally, there was not a documented
shown good results in some immunosuppressed patients and developed CMV disease. Although antigenemia assay has not been proven to be of clinical value for solid-organ transplantation, but correlation is inconsistent, and some investigators have considered it as important for patients with low/undetectable systemic viral load who can develop CMV disease. Although antigenemia assay has shown good results in some immunosuppressed patients and several institutions including ours, these results support the conclusion that CMV virus can develop CMV disease. To determine its role in these patients, it would have been necessary to measure CMV load in BAL samples and correlate these findings with those coming from traditional and shell vials cultures.

Our findings point to some issues that have to be studied in future research. First, it is required to clarify the real role of atypical germs and respiratory viruses in immunocompromised patients, and the contribution of microbiological cultures of TBB and PCR testing in BAL samples for diagnosing mycobacterial and fungal diseases. Second, because an invasive procedure such as a FB may be unsafe in some critically ill patients, it is important to evaluate the diagnostic accuracy of nonbronchoscopic BAL under these circumstances.

In conclusion, our findings highlight that as economical and epidemiological conditions of regions are different, systematical study of immunosuppressed patients with suspected pneumonia should be tried everywhere. Fortunately and accurate results are very useful for affected patients, clinicians, insurance companies and health authorities involved in care-giving activities and local guidelines development. Furthermore, in high incidence regions of tuberculosis, rapid identification and treatment of potential sources will allow better control of this kind of nosocomial-acquired infections.

Conflict of interest

Lázaro Vélez has received research funding from Astrazeneca and Roche Colombia, and has been a consultant for Pfizer. Other authors did not declare conflict of interest.

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