Rapid Diagnosis of Legionnaires' Disease by Bronchoalveolar Lavage

William R. Kohorst, M.D.; Steven A. Schonfeld, M.D.; James E. Macklin, M.D.; and Michael E. Whitcomb, M.D., F.C.C.P.

Legionella pneumophila has, in recent years, emerged as a common pulmonary pathogen in the normal and immunocompromised patient (ICP). Making a specific etiologic diagnosis of pneumonia in the latter group is a common clinical dilemma often complicated by poor specimen availability and risks of invasive procedures. Improved staining and isolation techniques for L pneumophila would suggest that early diagnosis could be possible if adequate specimens were available. This report summarizes our experience with bronchoalveolar lavage (BAL) with which we have diagnosed L pneumophila in eight immunocompromised patients, well in advance of more traditional methods. On the basis of this experience, we would advocate early BAL in the ICP as a rapid, safe, moderately sensitive and specific diagnostic test to aid in the identification of L pneumophila.

At the present time, most cases of Legionnaires’ disease are diagnosed retrospectively, long after therapeutic decisions must be made, by measuring indirect fluorescent antibody titers in acute and convalescent sera and applying accepted criteria for seroconversion. Since rising titers are not entirely specific, some patients who do not have the disease will be misdiagnosed by this approach. The lack of a highly sensitive technique for the rapid diagnosis of Legionnaires’ disease is usually not critical when evaluating immunocompetent patients since erythromycin, a relatively nontoxic antibiotic, can be used empirically in such patients. In fact, the great majority of normal patients with the disease will eventually recover, even if not treated.

However, the lack of a sensitive, rapid, and safe technique for diagnosing Legionnaires’ disease poses several critical problems, especially when evaluating immunocompromised patients suspected of having the disease. First, immunocompromised individuals with Legionella pneumophila pneumonia have a higher fatality rate than immunocompetent patients, making early diagnosis and therapy mandatory. Second, since a number of organisms must be considered in the differential diagnosis of pneumonia in these patients, the approach to empiric therapy is complex, and by necessity, must employ multiple antimicrobial agents, exposing patients with undiagnosed Legionnaires’ disease to potentially avoidable toxic drugs. Third, since thrombocytopenia or other coagulopathies often adds unacceptable risk to open or closed biopsy procedures, a relatively noninvasive diagnostic procedure would be highly desirable. In addition, the sensitivity of direct fluorescent antibody (DFA) stains of expectorated or suctioned sputum samples for L pneumophila may be relatively inadequate in patients with suspected Legionnaires’ disease. Although L pneumophila can be grown on artificial media from a variety of specimens, growth may take seven days or longer to detect, further delaying diagnosis. Also, obtaining adequate samples of lower respiratory tract secretions may be an additional problem.

Bronchoalveolar lavage (BAL) is a rapid and safe technique for sampling the distal airways and alveoli. We report here our recent experience with diagnostic segmental BAL in immunocompromised patients with pneumonia and the utility of this technique for diagnosing L pneumophila infection.

METHODS

Charts of all patients undergoing diagnostic flexible fiberoptic bronchoscopy (FFB) with BAL by The Ohio State University Hospitals Pulmonary Disease Division during the period of January 1, 1980, through January 31, 1982, were reviewed for the purpose of this study.

All patients underwent bronchoscopy with an Olympus BF B2 flexible fiberoptic bronchoscope (FFB) by the transnasal or endotracheal route. Xylocaine (1, 2 or 4 percent) was routinely used for local anesthesia of the upper airways prior to bronchoscopy in nonintubated patients. After introduction of the FFB, all attempts were made to avoid injection of xylocaine through the aspiration channel of the FFB until after BAL was completed. The FFB was wedged in a subsegment of resectional-lobectomy-involved lung, and BAL was performed by sequential installation, followed by slow syringe aspiration through the aspiration channel, of 50 ml aliquots of sterile saline solution. Approximately 20 to 30 ml was recovered from each 50 ml aliquot. Two or three separate 50 ml aliquots were usually employed for each segment lavaged.

A single 20 to 30 ml aliquot of BAL effluent was sent for L pneumophila studies; other aliquots were allotted for routine bacteriologic, cytologic, or pathologic studies. The BAL effluent was centrifuged at 3,000g for 30 minutes; the pellet was suspended in 0.5 ml of sterile saline solution and mixed with 4.5 ml of 0.2 M KC1/HCl acid wash reagent (pH 2.2). Then, 0.1 ml of this suspension was pipetted onto buffered charcoal yeast extract (BCYE) and BCYE containing cephalothin, colistin, vancomycin, and cyclohex-
Table 1—Summary of Patient Population

<table>
<thead>
<tr>
<th>Criteria for diagnosis</th>
<th>Patients Cultured</th>
<th>DFA only</th>
<th>DFA and culture</th>
<th>Serologic criteria</th>
<th>DFA, culture, and serologic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture only</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DFA only</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DFA and culture</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Serologic criteria</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

imide (Regional Media Laboratories, Panexa, KS) for isolation. Plates were incubated aerobically at 35 to 37°C without added CO₂ for up to two weeks. Growth consistent with *L. pneumophila* was subcultured onto plain BCYE, and blood agar and later confirmed by DFA staining.

The pellet of the centrifuged BAL effluent was also examined by the DFA staining technique using polyvalent antiserum to Legionella serogroups 1, 2, 3, and 4 (Centers for Disease Control, Atlanta). Sputum and other samples were examined by the same method. All smears were read by trained laboratory personnel unaware of the patient's clinical status other than the diagnosis of pneumonia; all smears were examined the same day that BAL was done. Smears with over five strongly fluorescing bacteria were reported as definitely positive; less than five but greater than zero were suspicious; and smears with zero strongly fluorescing organisms were considered negative.

A patient was considered to have Legionnaires' disease pneumonia if an acute pulmonary infiltrate developed accompanied by (1) a fourfold rise in convalescent serum immunofluorescent antibody (IFA) titer to ≥1:128 (two weeks or more after disease onset), or a single convalescent IFA titer of ≥1:512 in the appropriate clinical setting, or (2) demonstration of Legionnaires' organism by DFA staining of a fresh specimen and/or positive culture of lung tissue, pulmonary secretions, or BAL effluent in the absence of other demonstrable pulmonary pathogens.

RESULTS

Eighty-two patients underwent subsegmental BAL during the course of FFB. In 54 of these patients, BAL was performed as part of the investigation of a suspected pulmonary infection, and 37 of these 54 were considered to be immunocompromised (Tables 1 and 2). Nine of these patients fulfilled our criteria for diagnosis of Legionnaires' disease and their data are summarized in Table 3.

None of the remaining 28 immunocompromised patients who underwent BAL fulfilled our criteria for the diagnosis of Legionnaires' disease (see Methods). The BAL effluents in 18 of these 28 were examined with DFA staining and culture for *L. pneumophila*; all were negative. Bronchial washings were stained and cultured for Legionella in six of these 28 patients (two also had BAL effluent examined), and all were negative.

Direct fluorescent antibody stains of BAL effluent were reported as definitely positive for *L. pneumophila* in five patients and suspicious in one patient. Culture of the BAL effluent in five of these six patients grew *L. pneumophila*, serogroup 1. Only one patient (patient 2) with a positive or suspicious DFA stain of BAL effluent failed to grow *L. pneumophila* on culture; however, in patient 2, DFA staining of lung tissue for *L. pneumophila* at postmortem examination was strongly positive.

Three of the nine patients who fulfilled the criteria for Legionnaires' disease had negative DFA stains of their BAL effluents. However, cultures of the BAL effluent eventually grew *L. pneumophila*, serogroup 1, in two of these three patients. In these three patients with negative DFA-stained BAL smears, broad spectrum antibiotics had been administered prior to BAL; patient 4 received erythromycin for six days, patient 7 received ampicillin, clindamycin, and tobramycin for 17 days, and patient 8 received cephalothin, clindamycin, and gentamicin for two days preceding bronchoscopy. In contrast, of the patients with positive or suspicious DFA smears, only patient 2 (ticarcillin and tobramycin for six days) and patient 5 (tobramycin, clindamycin, and cefotaxime for two days) received any antibiotics for longer than one day prior to FFB and BAL.

No other specimens in any patients obtained within two days of BAL (sputum, bronchial washings, or transbronchial biopsy) were positive by DFA staining. In two patients (3 and 6), cultures of bronchial washings grew *L. pneumophila* (after six and three days, respectively); in one patient (patient 5), a culture of expectorated sputum obtained the day of BAL was positive after 13 days; and a transbronchial biopsy in patient 9 obtained the day of BAL was reported positive for *L. pneumophila* four days later. None of these culture results became positive prior to those of the BAL.

Table 2—Underlying Conditions in All 37 Immunocompromised Patients Undergoing BAL

<table>
<thead>
<tr>
<th>Condition</th>
<th>Confirmed Legionnaires</th>
<th>No Legionnaires</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's disease</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Chemotherapy for metastatic carcinoma</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Chronic renal failure, on hemodialysis</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Chronic corticosteroid therapy</td>
<td>9</td>
<td>28</td>
</tr>
</tbody>
</table>

Deaths of patients caused by *L. pneumophila* were not diagnosed promptly in 15 of 18 cases. However, culture of BAL effluent was diagnostic in five patients.
Table 3—Summary of Clinical Data in Patients with Legionnaires’ Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Sex</th>
<th>Primary Diagnosis</th>
<th>DFA Stain*</th>
<th>Culture↑ of BAL</th>
<th>Serum IFA Tilters†</th>
<th>Other‡ Specimens for Legionella</th>
<th>Antibiotics before BAL</th>
<th>Days-Infiltrate to BAL</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25, M</td>
<td>Acute myelogenous</td>
<td>+</td>
<td>ND</td>
<td>1:64 (1,2)</td>
<td>Sputum DFA –</td>
<td>PCN, TOB</td>
<td>1</td>
<td>Died</td>
<td>Post-mortem Lung DFA + Pseudomonas sepsis</td>
</tr>
<tr>
<td>2</td>
<td>56, M</td>
<td>Lymphocytic</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7</td>
<td>Died</td>
<td>Post-mortem Lung DFA + respiratory failure</td>
</tr>
<tr>
<td>3</td>
<td>45, M</td>
<td>Metastatic lung</td>
<td>+</td>
<td>ND</td>
<td>1:64 (1-4)</td>
<td>BW DFA –</td>
<td>CFX, ECN, SFX, TMP</td>
<td>3</td>
<td>Died</td>
<td>Post-mortem Lung DFA – cardiac arrest</td>
</tr>
<tr>
<td>4</td>
<td>21, M</td>
<td>Renal transplantation</td>
<td>–</td>
<td>ND</td>
<td>1:64 (1-4)</td>
<td>Sputum NA</td>
<td>ECN, CFZ, GEN</td>
<td>1</td>
<td>Recovered</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>42, M</td>
<td>Chronic renal failure</td>
<td>+</td>
<td>ND</td>
<td>1:2050 (1)</td>
<td>Sputum NA</td>
<td>TOB, CLI, CTX (2)</td>
<td>2</td>
<td>Recovered</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32, M</td>
<td>Dermatomyositis, corticosteroids</td>
<td>+ –</td>
<td>ND</td>
<td>1:256 (1)</td>
<td>Sputum NA</td>
<td>BW, DFA – Culture + (3)</td>
<td>1</td>
<td>Recovered</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>60, M</td>
<td>Acute myelogenous leukemia</td>
<td>–</td>
<td>ND</td>
<td>1:8100 (1)</td>
<td>ND</td>
<td>AMP, CLI, TOB (18)</td>
<td>7</td>
<td>Recovered</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>62, F</td>
<td>Postnecrotic cirrhosis</td>
<td>–</td>
<td>ND</td>
<td>1:2050 (1)</td>
<td>Sputum DFA –</td>
<td>CPH, CLI, GEN (2)</td>
<td>1</td>
<td>Died</td>
<td>Hepatic failure</td>
</tr>
<tr>
<td>9</td>
<td>49, F</td>
<td>Oat cell carcinoma</td>
<td>+</td>
<td>ND</td>
<td>1:128 (1-4)</td>
<td>TBBx</td>
<td>CFM, ECN, TOB (1)</td>
<td>1</td>
<td>Recovered</td>
<td></td>
</tr>
</tbody>
</table>

*Direct fluorescent antibody stain of bronchoalveolar lavage: + is positive; +/ –, suspicious; and –, negative.
†Numbers in parentheses are L. pneumophila serogroups; ND, not done.
§Numbers in parentheses are days till culture reported positive.
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†Numbers in parentheses are L. pneumophila serogroups; ND, not done.
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**Discussion**

Bronchoalveolar lavage has become recognized as a powerful tool for directly assessing the alveolar milieu in various lung diseases. Analysis of the BAL cell pellet can suggest the correct diagnosis and provide insight into the intensity of the inflammatory response in sarcoidosis, hypersensitivity pneumonitis, and fibrosing alveolitis and can be diagnostic in alveolar proteinosis and alveolar cell carcinoma.\(^{9,10}\) Moreover, many infectious diseases including blastomycosis, tuberculosis, histoplasmosis, coccidioidomycosis, cryptococcosis, aspergillosis, cryptoccocal inclusion disease, and *Pseudomonas carinii* infection have been diagnosed by performing appropriate smears and cultures of the BAL effluent.\(^{11,12}\) A single case of Legionnaires’ disease diagnosed by DFA staining of BAL effluent has been reported previously.\(^3\) The DFA-positive material has also been obtained via percutaneous lung aspiration\(^{13}\) and transtracheal aspiration,\(^{14}\) but these techniques may be hazardous in an immunocompromised host, especially one with thrombocytopenia, hypoxemia, or coagulopathy. Enzyme-linked immunosorbent assay (ELISA) for Legionella antigen in urine appears to be a sensitive and specific technique for the rapid diagnosis of Legionnaires’ disease, but is not yet widely available.\(^{15}\)

The usefulness of BAL in diagnosing Legionnaires’ disease depends upon the sensitivity and specificity of the fluorescent antibody preparation used to stain the lavage effluent. Cherry et al\(^7\) have documented the specificity of the DFA stain for *L. pneumophila*. While the likelihood of cross reactivity appears remote, the possibility of an occasional false-positive stain must be recognized.\(^7,16\) In only one patient with a positive or suspicious DFA stain was the culture negative. We doubt that this may have been a false-positive speci-
men because of the large numbers of DFA staining organisms consistent with *L pneumophila* seen on postmortem lung sections. In addition, the BAL effluents obtained in 18 of the 28 patients who did not have Legionnaires' disease pneumonia, were all negative for *L pneumophila* by both DFA staining and culture, further confirming specificity of this technique in this population.

At first glance it may appear that DFA staining of BAL effluent does not offer any advantage in sensitivity over examination of expectorated or suctioned lower respiratory tract secretions. Winn et al report finding positive or suspicious smears for Legionella in eight out of ten sputum or transtracheal aspirate samples from patients with confirmed Legionnaires' disease. Edelstein et al report a sensitivity of 50 percent in DFA staining of respiratory tract specimens. The BAL in our study yielded positive or suspicious smears in six of nine patients, a sensitivity rate similar to the reports mentioned above. However, in seven of our nine patients, sputum stains were either negative prior to bronchoscopy, or adequate specimens of lower respiratory tract secretions could not be obtained prior to bronchoscopy. Bronchoscopy was undertaken only after specimens obtained by less invasive techniques failed to yield a diagnosis.

In the three patients with negative DFA stains of BAL effluent, we feel that prior prolonged administration of broad-spectrum antibiotics may have contributed to the absence of organisms seen on the initial DFA stained smear. However, the diagnosis of *L pneumophila* pneumonia was later confirmed in two of them by culture of the BAL effluent (positive cultures after two and six days), still providing a specific diagnosis within a relatively brief time. The third patient met seroconversion criteria, and of note, had BAL performed 18 days after antibiotics were begun, and seven days after infiltrates first appeared on chest roentgenograms. However, we found that administration of broad-spectrum antibiotics for short periods of time (24 to 48 hours) did not seem to adversely affect the results of the bronchoalveolar lavage DFA stains. Thus, it appears that appropriate empiric broad spectrum antibiotic therapy need not be withheld when *L pneumophila* infection is suspected if early treatment is felt necessary and diagnostic BAL must be delayed. This concept is supported by the observations of Edelstein and co-workers that DFA stains of respiratory tract secretions may still be diagnostic at least up to 48 hours after specific antimicrobial therapy is started.

We have come to recognize *L pneumophila* as a major cause of opportunistic infection (nosocomial and community acquired) in our hospital in the last 2½ years, perhaps increasing the likelihood that BAL will yield this particular diagnosis in our immunocompromised population and contributing to our unique experience. Nevertheless, since BAL can be performed rapidly, safely, and without contraindication at the bedside, even under adverse clinical circumstances, we would advocate this technique be used early in the evaluation of immunocompromised patients with pneumonia, particularly with a clinical picture consistent with *L pneumophila* infection. However, since BAL effluent may not yield DFA or culture-positive results in all cases of confirmed *L pneumophila* pneumonia, particularly when prior antibiotic administration has occurred, a negative BAL must be interpreted cautiously in light of clinical presentation, serologic studies, and biopsy specimens as they are available.

In conclusion, we feel our experience warrants the inclusion of DFA staining of BAL effluent in the early evaluation of the immunocompromised patient with suspected Legionnaires' pneumonia, especially in those in whom recovery of adequate samples of lower respiratory secretions via noninvasive techniques proves difficult or impossible. Such an approach may improve our ability to confirm specific etiology and constrain purely empiric and potentially toxic antibiotic therapy.

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REFERENCES

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Pediatric Radiology

The Department of Radiology, The Children's Hospital Medical Center, Harvard Medical School, will present the Annual Course in Pediatric Radiology at the Hyatt Regency Hotel, Cambridge, October 31-November 2. For information, contact Educational Resources Associates, Inc., PO Box 369, Brookline, Massachusetts 02146 (617:738-8859).

Myocardial Revascularization in Acute Conditions

The European Society of Cardiology will present this symposium at the Sheraton Hotel, Brussels, Belgium, November 2-5. For information, contact Mrs. R. Fonteyne, Information Medicale Express, Avenue du Vossegat 16, B-1180 Brussels, Belgium.

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The Second International Conference on Electrophysiologic Basis for Diagnosis and Management of Cardiac Arrhythmias will be held at the Performing Arts Center, Milwaukee, October 6-8. The conference is sponsored by Mount Sinai Medical Center, Milwaukee and the University of Wisconsin-Extension, Continuing Medical Education. For information, contact Ms. Sarah Z. Aslakson, 465 WARF Building, 610 Walnut Street, Madison, Wisconsin 53705 (608:263-2856).