

Diagnosis of Legionnaires' disease from transbronchial lung biopsy using the fiberoptic bronchoscope

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Legionnaires' disease is a recently described illness with findings similar to those of other pneumonias.¹⁻³ The diagnosis is usually made by demonstrating a fourfold rise in titres of antibodies to the gram-negative bacterium responsible for the disease — *Legionella pneumophila*. However, there are also elevated titres of antibodies to this agent in other diseases, namely tularemia, leptospirosis and plague.⁴ Lung biopsy tissue has recently been used to diagnose this disease with the aid of direct fluorescent antibody staining,⁵ but false-positive results have occurred with other bacteria found in the sputum, including strains of *Pseudomonas fluorescens*.⁵ Direct fluorescent antibody staining has also been used to demonstrate *L. pneumophila* in the sputum.⁶ There have been very few reports of the isolation of this organism from living persons.⁷ Lung tissue may also be examined with the use of the Dieterle silver impregnation method or by the Wohlbach modification of the Giemsa stain.^{8,9} Lattimer, McCrone and Galgon¹⁰ have reported the isolation and identification of *L. pneumophila* from a transtracheal aspirate.

Fiberoptic bronchoscopy, since its introduction by Ikeda in 1966, has proved to be a safe method of obtaining lung tissue for both staining and culture.^{11,12} It has been used in the diagnosis of slowly resolving pneumonias and pulmonary infiltrations of unknown cause.^{13,14} In this paper we describe a case of progressive pneumonia in which transbronchial lung biopsy helped in the final diagnosis of Legionnaires' disease; to our knowledge this is the first

report in which this procedure has helped to confirm the diagnosis of Legionnaires' disease in a patient with pneumonia of undetermined cause. We recommend that a transbronchial lung biopsy be done in cases of pneumonia that does not respond to therapy or early in the course of severe pneumonia when the cause is obscure.

Case report

Clinical course

A 52-year-old alcoholic woman was admitted to hospital in August 1978 because of right lower lobe pneumonia. Increasing shortness of breath and a cough productive of green sputum had begun approximately 2 weeks earlier. A few days prior to admission she had first noted general malaise, weakness and muscle aches. There had been no diarrhea, and her health had previously been good.

She appeared to be acutely ill. Her pulse rate was 120 beats/min, blood pressure 130/80 mm Hg and temperature 39.5°C. The only significant physical findings were in the chest: dullness to percussion, increased tactile fremitus and crepitations over the right lower lobe of the lung.

A chest roentgenogram revealed right lower lobe consolidation. The hemoglobin level was 10.6 g/dl and the leukocyte count $9.1 \times 10^9/l$ (83% neutrophils, 11% lymphocytes and 5% band cells, with evidence of toxic granulation of the neutrophils). Gram-staining of the sputum showed more than 10 epithelial cells and fewer than 25 pus cells per high power field and normal oral flora. Arterial blood gas values were as follows: pH 7.53, carbon dioxide tension 28 mm Hg and oxygen tension 50 mm Hg.

The patient was admitted, and therapy with oxygen and penicillin G, 1 million units given intravenous-

ly every 6 hours, was begun. Over the next 2 days there was no clinical improvement, and on the third day after admission the antibiotic therapy was changed to cephalothin, 1 g given intravenously every 4 hours. Two days later there was clinical deterioration, with worsening of the blood gas values and chest abnormalities, and radiologic evidence of bilateral pneumonia. She was transferred to the intensive care unit, where an endotracheal tube was inserted and ventilation begun. Gentamicin, 80 mg given intravenously every 8 hours, was added to the antibiotic regimen. On the seventh day after admission there was no evidence of improvement and the patient was referred to the respiratory service for fiberoptic bronchoscopy. Bronchial washings and transbronchial biopsies were done in the right lower lobe. Therapy with isoniazid, 300 mg/d, and streptomycin, 1 g/d, was started while the results of the bronchoscopy were awaited.

The following day the antituberculous medications and other antibiotics were discontinued, and therapy with erythromycin, 500 mg intravenously every 6 hours, was begun. Within 24 hours the patient became afebrile, and progressive clinical improvement along with radiologic clearing of the pneumonia occurred.

Bacteriologic findings

Cultures of sputum and transbronchial washings were negative for bacterial pathogens, including *L. pneumophila*, which was sought on chocolate agar and Thayer-Martin medium. Smears and cultures for fungi and mycobacteria were also negative. Serologic testing for influenza viruses, adenoviruses and *Mycoplasma* showed no significant rise in antibody titres, but titres of antibodies to *L. pneumophila*, measured by the indirect fluorescent anti-

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body technique at the Ontario Provincial Laboratory, were 1:128 the day the patient became afebrile and 1:4096 3 weeks later.

Biopsy results

The transbronchial biopsy specimen showed changes of pneumonia. The alveolar spaces were filled with a fibrinous, proteinaceous exudate containing many macrophages and a few neutrophils. There was some sloughing of alveolar lining cells. The alveolar walls were widened by edema, slight fibrosis and a mild infiltrate of mononuclear inflammatory cells (Figs. 1 and 2). The use of special stains for microorganisms gave negative results. Because the clinical and biopsy findings conformed to those described for Legionnaires' disease, Dieterle staining was done, but its results were read as negative.^{15,16} However, the paraffin block was sent to the Center for Disease Control in Atlanta, Georgia, where indirect immunofluorescent staining of sections gave positive results for the Knoxville strain of *L. pneumophila*.

Discussion

Legionnaires' disease is a recently described cause of pneumonia that is usually diagnosed retrospectively.¹ Some features may help in differentiating it from other types of pneumonia; these include a pro-

dromal viral illness, a dry cough, confusion, diarrhea, lymphopenia, neutrophilia and hyponatremia.^{3,17,18} The organism is a gram-negative bacterium that is difficult to grow. As a result, serologic methods and immunofluorescent staining of autopsy material have been used in the diagnosis of the disease.^{5,8}

Lattimer and colleagues¹⁰ have described the isolation of *L. pneumophila* from a transtracheal aspirate. Fiberoptic bronchoscopy, an alternative to transtracheal aspiration, yields tissue as well as bronchial secretions, and has been used to diagnose other types of pneumonias, such as that caused by *Pneumocystis carinii*. The morbidity of the procedure is low, and open lung biopsy is avoided.¹⁹ In our patient transbronchial biopsy yielded enough tissue for staining by the Dieterle method and by indirect immunofluorescence. Even though there was a successful outcome in this case, earlier transbronchial biopsy might have been used to make the diagnosis.

The light microscopic appearance of the biopsy specimen, while not specific for Legionnaires' disease, was consistent with it, and this plus the clinical findings led to its further examination for specific evidence of Legionnaires' disease. The negative results of Dieterle staining were probably due to poor technique, since even in retrospect we

could not make a positive diagnosis from this one section. Negative results of Dieterle staining have been found in biopsy specimens from other patients with serologically proven disease, but usually the direct immunofluorescent test has also given negative results. Recently a transbronchial biopsy specimen from a patient with serologically proven Legionnaires' disease gave negative results with direct immunofluorescent staining.⁶

In summary, transbronchial biopsy may be useful in cases of Legionnaires' disease in making an early diagnosis if bronchial secretions and lung tissue are examined by immunofluorescent staining.

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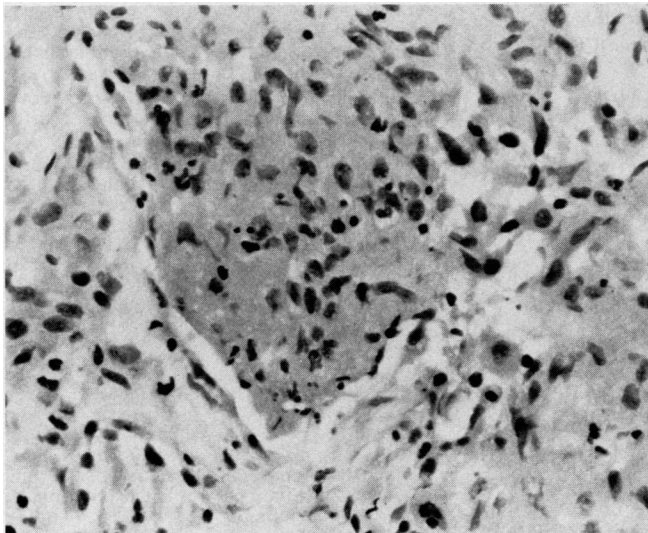


FIG. 1—Alveolar spaces filled with fibrinous exudate, many macrophages and few neutrophils (hematoxylin-eosin [H-E]; $\times 400$, reduced 40%).

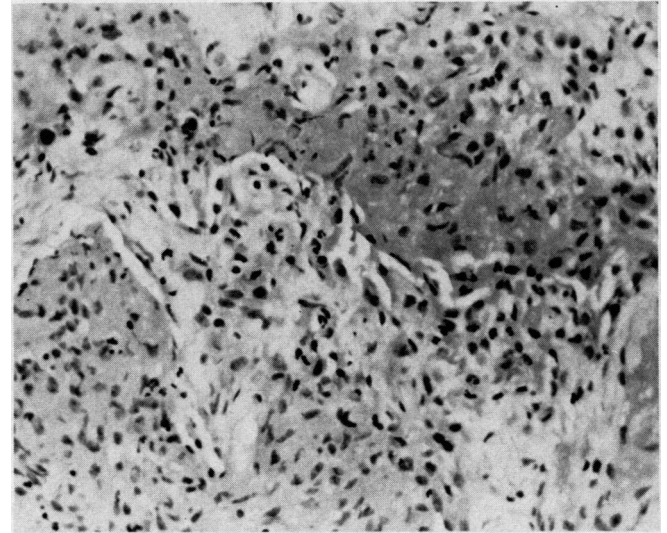


FIG. 2—Edematous alveolar walls contain mononuclear inflammatory cells and occasional neutrophils (H-E; $\times 250$, reduced 40%).

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