A Pseudoepidemic of Legionella Infections*

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During a six-month period, we observed an increase in the incidence of presumed Legionnaires' disease (LD) due to false-positive direct immunofluorescent antibody (DFA) staining. Contamination of the DFA staining reagents with Legionella appeared to account for our pseudoepidemic. Although a positive DFA stain has been regarded as highly specific for the diagnosis of LD, the clinician must interpret such results with caution.

A positive Legionella DFA result is usually enough evidence to treat a patient with a full course of erythromycin. At Henry Ford Hospital, as well as other centers, the Legionella DFA stain has been a very valuable diagnostic test.1,2 However, during a recent six-month interval, the test's specificity became suspect: Legionnaires' disease (LD) was diagnosed much more frequently than usual and the clinical presentations were often very atypical, leading us to suspect a pseudoepidemic. We reviewed the medical records of all patients at Henry Ford Hospital who had positive Legionella DFA tests from July to December, 1982, and identified patients without a clinical Legionella infection. Patients were included in our series if they had a positive DFA test and met one of the following criteria: 1) no clinical pneumonitis; 2) respiratory symptoms causally linked to another process; 3) highly atypical LD presentation with convalescent indirect immunofluorescent antibody (IFA) titers <8; or 4) a persistently positive sputum DFA stain despite a full course of therapy with erythromycin.

Because we suspected that Legionella in the hospital water supply was responsible for our pseudoepidemic, we took sample specimens from hospital showers and sinks at monthly intervals. The DFA reagents were also tested for DFA organisms. In addition, we attempted to isolate Legionella from hospital water specimens utilizing buffered charcoal yeast-extract (BCYE) agar.

We attempted to recover Legionella from lung tissue in one patient (case 2) using BCYE agar.

In the DFA staining method,1 clinical specimens are first screened with polyvalent fluorescein-conjugated Legionella antibodies obtained from the Centers for Disease Control, Atlanta, Georgia and, when positive, specimens are stained with each of the monovalent specific conjugates to determine the species and serotype. Conjugates utilized include the following organisms: L pneumophila serogroups 1 to 6, L micdadei, L bozemani, L dumoffii, L gormanii, L longbeachae serogroups 1 and 2.

The findings for the eight patients are summarized in Table 1. Only four patients had clinical pneumonitis, and three patients (1, 3 and 4) received antibiotic therapy judged effective for LD. All patients survived. The only attempt to recover Legionella organisms was in case 2 where lung tissue was available, but isolation of Legionella was not possible.

On several occasions, water specimens from hospital showers and sinks grew numerous fluorescent organisms staining with the conjugate to L pneumophila serogroup 3. In January 1983, after six months of suspicious results, all Legionella DFA reagents were replaced. The phosphate-buffered saline reagent used during this six-month period was found to stain positive with DFA conjugate to L pneumophila serogroup 3. After the reagents were replaced, the incidence of positive DFA results dropped considerably, and no further cases of LD were reported during the next four months.

Since 1977, the DFA method for staining Legionella has been a reliable diagnostic tool at Henry Ford Hospital.1,3 During the last six months of 1982, the incidence of Legionella infection doubled with all but two cases occurring in immunocompetent hosts. With many of these cases occurring during colder months we questioned the validity of our DFA results. Invalid indications for requesting the DFA test were common; the DFA test was indicated only in two patients of this series.

The positive DFA results in our patients may have occurred through several mechanisms: 1) bacteria with cross-reacting antigens to Legionella, 2) oropharyngeal colonization with Legionella organisms, or 3) contamination of the DFA staining reagents with Legionella. Many Gram-positive cocci in the patient's respiratory secretions may fluoresce with the DFA method because the conjugate is prepared from rabbit sera which may contain natural antibodies directed against common environmental organisms in addition to the Legionella.4

Several bacteria which fluoresce strongly share similar morphology and antigens with Legionella species. One strain of Pseudomonas fluorescens is known to cross-react with the DFA conjugate for L pneumophila serogroup 1. In our series, the persistently positive sputum DFA stain in patient 3 was possibly due to oropharyngeal colonization of Pseudomonas fluorescens. Three strains of Bacteroides fragilis and one strain of Pseudomonas alcaligenes have also been reported to weakly fluoresce with the DFA conjugate.5 Since B fragilis may be found in 15 to 20 percent of transtracheal aspirate specimens from patients with aspiration pneumonia, patient 4 could have false-positive DFA tests on the basis of cross-reactivity with B fragilis.6

Asymptomatic oropharyngeal colonization with Legion-

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Pseudoepidemic of Legionella (Ristagno, Saravolatz)
Table 1—Summary of Data from Eight Patients with False-positive Legionella DFA Test Results

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>History</th>
<th>Roentgenogram</th>
<th>DFA*</th>
<th>Acute</th>
<th>Convalescent</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>chest pain, yellow sputum</td>
<td>diffuse reticulo-nodular infiltrates</td>
<td>&gt;25 <em>L. pneumophila</em> serogroup 6; <em>L. bozemanii</em>; <em>L. longbeachae</em> serogroup 2 (sputum)</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>Mycoplasma pneumonia, CF titer = 1:32,768</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>chronic dyspnea</td>
<td>basilar interstitial infiltrates</td>
<td>&gt;5 polyvalent conjugate (lung biopsy)</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>usual interstitial pneumonia; dermatomyositis congestive heart failure</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>idiopathic thrombocytopenia purpura; orthopnea</td>
<td>chronic interstitial infiltrates</td>
<td>&gt;25 <em>L. pneumophila</em> serogroup 1 (sputum and <em>Pseudomonas fluorescens</em> colonies isolated from sputum)</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>non-productive cough</td>
<td>left basilar infiltrate</td>
<td>&gt;25 <em>L. pneumophila</em> serogroup 6; <em>L. gormanii</em> (sputum)</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>aspiration pneumonia; polymyositis</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>Alport’s syndrome; recent renal transplantation</td>
<td>left basilar infiltrate</td>
<td>50 polyvalent conjugate (sputum)</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>pneumococcal pneumonia</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>intravenous drug abuse; chest pain, bloody sputum asthma; acute dyspnea</td>
<td>multiple cavity nodules</td>
<td>15 <em>L. pneumophila</em> serogroup 6 (sputum)</td>
<td>&lt;8</td>
<td>—</td>
<td><em>Staphylococcus aureus</em> endocarditis with septic pulmonary emboli asthma exacerbation</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>acute pulmonary edema and respiratory failure, resolved</td>
<td>normal</td>
<td>10 polyvalent conjugate (sputum)</td>
<td>&lt;16</td>
<td>—</td>
<td>no pneumonia; DFA obtained after resolution of pulmonary edema</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>acute pulmonary edema and respiratory failure, resolved</td>
<td>no infiltrates</td>
<td>10 polyvalent conjugate (sputum)</td>
<td>&lt;8</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*Direct immunofluorescent antibody (DFA); number of fluorescent organisms per slide
†Indirect fluorescent antibody (IFA)

ella organisms has been described recently in asymptomatic hospital employees and immunosuppressed patients.4 The presence of Legionella in the hospital’s potable water supply and respiratory treatment devices could account for DFA-positive organisms from the oropharynx of these individuals. During our LD pseudoepidemic, specimens from hospital showers and sinks revealed numerous fluorescent organisms stained by *L. pneumophila* serogroup 3 DFA conjugate. Although it is possible that Legionella in the water supply would result in widespread patient oropharyngeal colonization and subsequent false-positive DFA sputum results, *L. pneumophila* serogroup 3, was a rare DFA-positive result during the LD pseudoepidemic.

Typical fluorescent organisms stained with Legionella DFA conjugate were present in the phosphate-buffered saline DFA reagent used during the last six months of 1982. In January 1983, coincident with the replacement of all Legionella DFA reagents and the absence of Legionella organisms in the hospital water supply, DFA sputum-positive results were not reported for four months. We believe that our cluster of LD cases was a pseudoepidemic possibly caused by laboratory contamination of sputum with reagents used in performing the DFA test.

The increased incidence of uncommon pathogens frequently raises the question of factitious microbiologic data, as was the case in this first reported pseudoepidemic of Legionella.5 When laboratory data for a diagnosis for LD do not corroborate clinical findings, a pseudoepidemic such as described here should be suspected so that unnecessary antimicrobial therapy is avoided.

REFERENCES