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Legionella feeleii Serotype 2 Pneumonia in a Man with Chronic Lymphocytic Leukemia: a Challenging Diagnosis▽

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***Legionella feeleii* has rarely been reported as causing pneumonia in patients with hematologic malignancies. We present a case of *Legionella feeleii* serotype 2 pneumonia with empyema in a man with chronic lymphocytic leukemia and describe the methods of identifying this organism using both standard methods and newer diagnostic techniques.**

CASE REPORT

A 53-year-old white man presented to the National Institutes of Health, Bethesda, MD, to start his fourth cycle of fludarabine and rituximab for chronic lymphocytic leukemia (CLL). He had been diagnosed with CLL in 1998 but had only been started on chemotherapy 3 months prior to this admission when he experienced fatigue, anorexia, progressive lymphadenopathy, and a new left pleural effusion. On this presentation to the hospital, the patient complained of 7 days of fevers (39°C to 40°C), chills, a dry nonproductive cough, sharp right-sided lower back pain when lying down, and drenching night sweats. He denied diarrhea and had had no sick contacts. He had had a dental cleaning 7 days prior to the onset of these symptoms. He lived in Montana, where he worked in an office-based job in the construction industry, was a nonsmoker, had no pets, and had never received either a pneumococcal or influenza vaccine.

The patient was nontoxic appearing and able to speak in full sentences. His temperature on admission was 38.4°C, blood pressure 115/69 mm Hg, heart rate 102 beats per minute, and respiratory rate 20 breaths per minute, with an oxygen saturation of 95% on ambient air. His examination was unremarkable, with clear breath sounds bilaterally without rhonchi or wheezes. He had mild leukopenia (white blood cell count of 2,800 cells/μl, 69% neutrophils, 14% lymphocytes, and 13% monocytes), mild anemia (hemoglobin, 9.7 g/dl), and moderately elevated liver enzymes (aspartate aminotransferase, 58 IU/liter; alanine aminotransferase, 82 IU/liter). Immunoglobulins were low (IgG level, 323 mg/dl; IgA, 21 mg/dl; IgM, 14 mg/dl). A computed tomography (CT) scan of the chest showed bilateral hilar lymphadenopathy, a small left-sided pleural effusion, airspace disease in the right lower lobe with loculated effusions, and an area of cavitation (Fig. 1).

Bronchoscopy revealed scant white secretions, and the bronchioalveolar lavage (BAL) fluid showed moderate neutrophils, few Gram-negative bacilli, no acid-fast or modified acid-fast bacilli, and a few budding yeast. A thoracentesis yielded cloudy, amber pleural fluid. The pleural fluid showed a white blood cell count of 8,350 cells/μl (65% neutrophils), a glucose level of 80 mg/dl, a lactate dehydrogenase level of 945 U/liter, and a total protein level of 3.5 g/dl, consistent with an exudative effusion. After the procedures, ceftriaxone and azithromycin were initiated for community-acquired pneumonia. The patient became afebrile within 24 h of admission and did not require any supplemental oxygen. In-house real-time PCR performed on the BAL fluid was negative for both *Legionella pneumophila* serogroups 1 to 16 (*mip* gene) and *Pneumocystis jirovecii* (major surface glycoprotein gene). Routine bacterial, fungal, *Nocardia*, *Legionella*, and respiratory virus cultures of the BAL fluid remained sterile. A repeat CT scan 48 h after admission showed a significant worsening of bilateral pleural effusions. Ceftriaxone and azithromycin were discontinued, and meropenem was initiated for a possible aspiration during his dental procedure.

On day 5 of hospitalization, there was growth of Gram-negative bacilli on buffered charcoal yeast extract (BCYE) medium from the left pleural fluid, consistent with *Legionella*. The isolate did not grow in sheep blood agar. Testing of the patient isolate using the *Legionella* Poly-ID polyvalent fluorescent antibody (IFA) test (Remel, Lenexa, KS) for 31 serogroups of *Legionella* was nonreactive. The isolate was not amplified by the in-house PCR for *L. pneumophila* serogroups 1 to 16. Bacterial 16S rRNA sequencing at the 500- and 1,500-bp levels (Microseq, Applied Biosystems, Foster City, CA) was performed and showed a 99.6% match with GenBank sequences of *L. feeleii.* Because 16S rRNA sequencing cannot distinguish between *L. feeleii* serogroups 1 and 2, analysis of the transfer DNA (tDNA) intergenic spacer region was performed as described by De Gheldre et al. (3). Compared to data for the *L. feeleii* serogroup 1 type strain (ATCC 35072), the tDNA results indicated the patient isolate and the *L. feeleii* serogroup 2 reference strain (ATCC 35849) differed by a single base. We did not consider this difference to be significant

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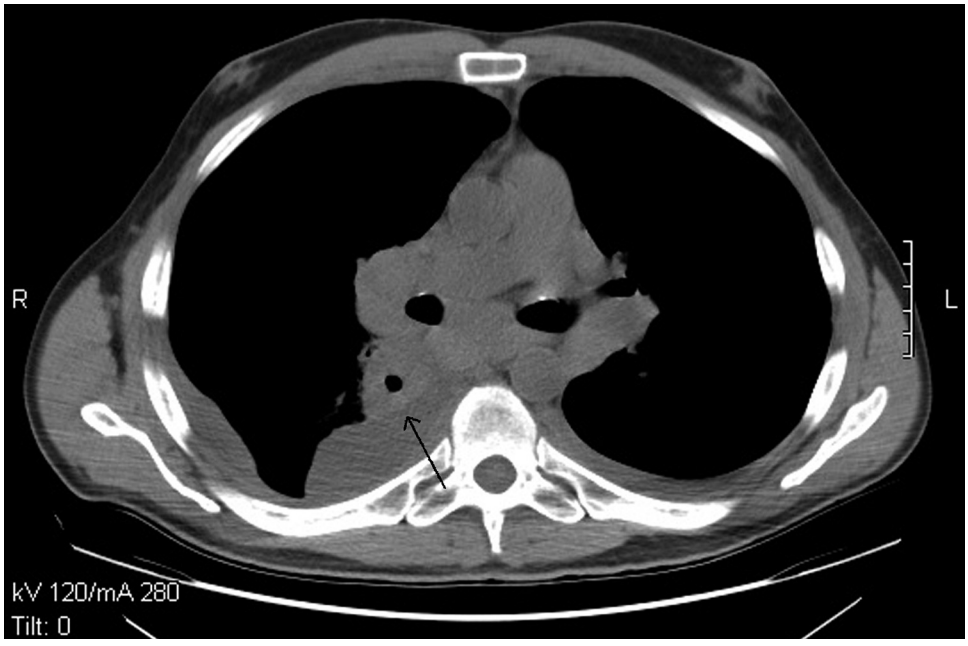

FIG. 1. CT scan of the chest showed bilateral hilar lyphadenopathy, a small left-sided pleural effusion, airspace disease in the right lower lobe with loculated effusions, and an area of cavitation (black arrow).

enough to definitively identify the patient's isolate as *L. feeleii* serogroup 2. The *L. feeleii* serogroup 1 type strain, the *L. feeleii* serogroup 2 reference strain, and the patient's isolate were compared using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with an Autoflex III instrument (Bruker Daltonics, Inc., Billerica, MA). Several colonies of each organism were suspended in 100% ethanol, and the extraction of bacteria was performed as described by Stevenson et al. (26). Spectra generated from multiple spots gave consistent results and demonstrated the patient isolate to have a spectrum pattern identical to that of the reference strain of *L. feeleii* serogroup 2.

Antibiotics were changed to intravenous azithromycin (500 mg daily), and the patient underwent tube thoracostomy drainage. The hospital course was complicated by the development of a chylothorax, which necessitated pleurodesis. The patient made a full recovery after 2 weeks of intravenous azithromycin and was discharged to complete one further week of oral therapy at home. We were not able to determine the source of the patient's *Legionella* infection.

There have been at least 50 *Legionella* species and 70 serogroups identified. *L. feeleii* was first reported as the cause of an outbreak of Pontiac fever affecting 317 auto workers in Ontario, Canada, in 1981 (7). Symptoms included fever, headache, and severe muscle aches, but there was no evidence of pneumonia. A Gram-negative, rod-shaped organism was identified from a water-based coolant which on DNA hybridization was less than 10% related to previously known *Legionella* species. The organism was subsequently named *L. feeleii.* In 1985, a second serogroup of *L. feeleii* that was serologically distinct from the reference strain was identified as the cause of pneumonia in two patients (28).

Cases of non-*pneumophila Legionella* pneumonia have been found more frequently in the immunosuppressed (8, 10, 14, 19, 24), including a man with chronic lymphocytic leukemia (14). Only rarely have cases been reported in healthy individuals (27). Since the identification of *L. feeleii* in 1984 as the cause of Pontiac fever (7), there have only been 13 reported cases of *L. feeleii* pneumonia, of which 7 were community acquired (12, 14, 19, 24, 31), 3 were hospital acquired (8, 10, 12), and 3 were not clearly defined (27, 28). Mortality rates as high as 37.5% have been reported (10). The prevalence of *L. feeleii* pneumonia is difficult to determine, but it is presumably rare. Yu et al. found the incidence of community-acquired *L. feeleii* to be only 0.4% out of 508 cases of culture-confirmed community-acquired *Legionella* pneumonia in their multinational surveys (31). The initial case reports were all from the northern United States and Canada, suggesting a geographical predilection, but more recently cases have been described in a southern state (14) and internationally (12). Of all these reported cases of legionellosis, only three isolates have been identified as belonging to *L. feeleii* serogroup 2 (24, 28).

Pneumonia due to non-*pneumophila Legionella* species resembles that due to *L. pneumophila* clinically and radiographically. Ninety percent of patients are febrile, 50% of patients have a temperature of greater than 39.4°C (16), and small to moderate-size pleural effusions are common. Interestingly, in immunosuppressed patients, pulmonary infiltrates due to non-*pneumophila Legionella* species are often rounded or nodular and have a greater tendency to cavitate than in those patients with intact immune systems (17). The initial CT scan performed on our patient noted both of these findings. Chylotho-

rax has rarely been reported as a complication of lymphoma (18) and has not previously been associated with *Legionella* pneumonia. Although lymphomas account for three-quarters of the tumor-associated cases of chylothorax, it has been seen as a complication of CLL (1, 5, 32), which was the most likely cause in our patient.

Assays for detection of *Legionella* antigen in urine, including the BinaxNOW *Legionella* urinary antigen test (Inverness Medical, Bedford, United Kingdom), target only *L. pneumophila* serogroup 1. Direct fluorescent antibody (DFA) staining on respiratory secretions has a sensitivity ranging from 25 to 75% (6), with only a limited number of fluorescein-conjugated polyclonal sera available for *L. pneumophila* and non-*pneumophila* species. These reagents do not include antibodies reactive to uncommon *Legionella* species, such as *L. feeleii* serotype 2. False-positive results can occur due to cross-reactions with some bacteria found in respiratory specimens or *Legionella* bacteria present in buffers or wash solutions.

Use of PCR for direct specimen testing is appealing due to the rapidity of the results. Cloud et al. were able to detect 100% of culture-positive respiratory samples using PCR targeting the 16S rRNA gene, which is specific for *L. pneumophila*, and demonstrated 98% specificity (2). Real-time PCR for the macrophage infectivity potentiator (*mip*) gene, which is genus specific, can be performed on respiratory samples, but published assays detect only *L. pneumophila*. A real-time PCR described by Wilson et al. showed 100% sensitivity and specificity for all the *L. pneumophila* serogroup 1 to 14 isolates tested and was negative for all non-*pneumophila* isolates tested, although there were no isolates of *L. worsleiensis* and *L. fairfieldensis*, which would be expected to produce a positive result based on results of their BLAST search (29). Recently Yang et al. described a real-time PCR assay that targets the 23S-5S rRNA gene spacer region and can detect and differentiate between *L. pneumophila* and non-*pneumophila Legionella* species directly from patient specimens (30). This assay demonstrated a 100% sensitivity and specificity for *L. pneumophila* and identified 18 more positive specimens than did culture. Non-*pneumophila Legionella* species detected by this assay were identified by *mip* gene sequence analysis to be *L. longbeachae* ($n = 2$), *L. cincinnatiensis* ($n = 1$), and *L. micdadei* ($n = 1$), in addition to *Legionella* in seven samples in which *mip* sequence analysis suggested novel *Legionella* species. *Legionella feeleii* serogroup 2 would not be identified by this assay because the *mip* gene sequence does not distinguish between serogroups 1 and 2 (23).

Culturing *Legionella* species on BCYE agar has been the gold standard for the diagnosis of legionellosis and has a reported sensitivity of 20 to 95% depending on the severity of the illness (4, 13) and a specificity of close to 100% (4, 6, 13). However, Lee et al. showed that many non-*pneumophila* species have only marginal growth on BCYE agar, although both serogroups of *L. feeleii* have documented good growth (11). Two selective BCYE media with added polymyxin B, anisomycin, and either vancomycin (PAV) or cefamandole (PAC) are also available for use with nonsterile specimens, such as sputum. However, since *L. feeleii* is one of the few non-*pneumophila* species that does not produce ß-lactamase, its growth is hindered on the selective PAC medium (11). Therefore, sputum samples in patients with suspected legionellosis should be cultured on both BCYE agar and selective medium to increase the yield for both *pneumophila* and non-*pneumophila Legionella* species. Another disadvantage to relying on culture results is the slow rate of growth for many of the non-*pneumophila* species, as seen with our organism, which grew only after 5 days.

Identification of isolates of non-*pneumophila Legionella* species can also be challenging. The Legionella Poly-ID test kit that our laboratory used can identify 31 serogroups from 22 species of *Legionella*, but the test identifies only *L. feeleii* serogroup 1 and not serogroup 2, as was the case with our isolate. Commonly used PCR targets for speciation of isolates of *Legionella*, such as the 16s RNA gene, the *rpoB* gene, and the *mip* gene, cannot differentiate between serogroups of several species, such as *L. feeleii* and *L. hackeliae* (9, 23). MALDI-TOF MS can differentiate between bacterial strains through the analysis of their specific protein profiles. It has been demonstrated to be a rapid and accurate method for identifying bacterial isolates from patient specimens (25, 26). Moliner et al. recently applied MALDI-TOF MS to the identification of species of *Legionella* (15). Using an Autoflex II instrument (Bruker Daltonics, Inc., Billerica, MA) and no extraction of the bacteria before analysis, they were successful in differentiating between *Legionella* species but not between serogroups of species. The authors indicate that the failure to distinguish between serogroups of species may be due to their lack of extraction of the bacteria before analysis. Advances in MALDI-TOF MS instrument technology may also affect results. Using extraction of bacteria before analysis, the 7-year-old Ultraflex I instrument in our laboratory failed to distinguish between the serogroups of *L. feeleii*, but the latest instrument from Bruker Daltonics, the Autoflex III, combined with extraction of the bacteria before analysis, allowed us to clearly distinguish between serogroups 1 and 2 of *L. feeleii*.

Therapy for non-*pneumophila Legionella* species is similar to that for *L. pneumophila*. Azithromycin, 500 mg intravenously daily for 7 to 10 days, or levofloxacin, 500 mg intravenously daily for 10 to 14 days, is recommended (13, 20), but patients with underlying immunosuppression are often treated longer. Pedro-Botet et al. reviewed three observational studies that compared the clinical efficacy of macrolides with that of quinolones (21). These studies showed a shorter time to defervescence, a shorter length of hospitalization, and fewer complications, including pleural effusions, empyema, cavitation, and septic shock with quinolone use. However, none of these studies were randomized, and azithromycin was not used as one of the macrolide antibiotics. Interestingly, although it is not recommended for legionellosis, carbapenem monotherapy has been used successfully in the past (22).

This case underscores the importance of culture on BCYE medium in establishing the diagnosis of legionellosis. Real-time PCR with clinical samples is being used more frequently and should enable earlier pathogen-directed therapy. However, PCR has not been extensively studied for non-*pneumophila Legionella* species and will not identify certain non-*pneumophila Legionella* species. We highlight the challenges of diagnosing *L. feeleii* serogroup 2 using the current commercially available tests. With the more-widespread use of real-time PCR diagnostic methods and MALDI-TOF MS instrument technology, the diagnosis will become faster and more

accurate. These methods allow a suspicious colony of *Legionella* from a charcoal yeast extract plate to be directly identified to the species or serogroup level. Our patient made a rapid and full recovery with a 3-week course of azithromycin.

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