Osteomyelitis of the Patella Caused by *Legionella anisa*

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A 51-year-old man with a history of stage IV angioimmunoblastic T-cell lymphoma was diagnosed with osteomyelitis of the patella. *Legionella anisa* was identified by 16S rRNA gene sequencing and culture. The patient had pneumonia 2 months prior to this osteomyelitis episode. *L. anisa* was retrospectively detected in his lung tissue by 16S rRNA gene sequencing and was considered the source of the *L. anisa* that caused his patella osteomyelitis.

CASE REPORT

A 51-year-old man with a history of stage IV angioimmunoblastic T-cell lymphoma presented to the emergency department of Hackensack University Medical Center (Hackensack, NJ) with a 3-week history of right knee pain. The pain was associated with a decreased range of motion of the knee. He denied any other signs or symptoms, including fever, chills, or trauma. Five months prior to the reported onset of knee pain, he was found to have recurrence of the lymphoma, for which he underwent a left-sided tonsillectomy and was treated with alemtuzumab (anti-CD52 monoclonal antibody) and lenalidomide. Subsequently, 2 months prior to his current admission, he was also hospitalized with multifocal pneumonia and underwent a bronchoscopy during which a transbronchial lung biopsy specimen was obtained. The latter showed bronchiolitis obliterans with organizing pneumonia, and he was started on oral prednisone. Routine cultures of blood, sputum, and lung tissue for bacteria, mycobacteria, and fungi were negative. He was empirically treated with vancomycin at 1 g every 12 h, ceftazidime at 2 g intravenously every 8 h, azithromycin at 500 mg intravenously every 24 h, and voriconazole at 200 mg per os every 12 h. He recovered clinically with resolution of his pulmonary infiltrates as determined by a computerized axial tomography scan of the chest.

On physical examination, he was afebrile and thermodynamically stable. The right knee had no appreciable effusion but was tender over the anterior and lateral aspects of the patella, as well as the patellar tendon. The rest of his physical examination was unremarkable.

Magnetic resonance imaging of the right knee revealed moderate marrow edema and enhancement involving the anterior aspect of the patella. An indium whole-body scan revealed intense uptake of activity in the right patella. The patient underwent debridement of the patella. Gram staining of the tissue demonstrated numerous white blood cells, red blood cells, and large numbers of Gram-negative rods. He was initially treated with imipenem-colistin at 500 mg intravenously every 6 h. After *L. anisa* was identified (see microbiology investigation), the patient was treated for 8 weeks with moxifloxacin at 400 mg intravenously daily and had an uneventful course.

**Microbiology investigation.** A bone (patellar) biopsy specimen showed numerous extracellular and intracellular slender Gram-negative rods (Fig. 1). Routine aerobic and anaerobic cultures were negative. To rapidly identify the pathogen, DNA was extracted directly from the biopsy specimen with the MagneSiI Genomic Fix Tissue System (Promega, Madison, WI). Broad-range PCR primers capable of amplifying the first 5 to 525 bp of the 16S rRNA gene (1) were used to amplify the DNA, and the nucleotide sequence of the amplicon was determined. A 100% match with the *L. anisa* sequence in the GenBank database was obtained. After successful direct identification of the *L. anisa* nucleic acid from the biopsy specimen, culture was undertaken with buffered charcoal-yeast extract (BCYE) agar (BBL, Becton and Dickinson, Sparks, MD). Following incubation at 35°C in 5% CO₂, a pure culture of numerous white colonies appeared after 3 to 4 days. Gram staining (with safranin as a counterstain) revealed slender Gram-negative rods. Under long-wave UV light, the colonies exhibited a bright white-blue fluorescence. We have confirmed that the isolate grows only on BCYE agar and not on 5% sheep blood agar. 16S rRNA gene sequencing was performed, and a 100% DNA sequence match with *L. anisa* was obtained.

In the context of the identification of *L. anisa* from the patella, the patient’s recent presentation with pneumonia was reassessed. The pathology report had noted acute bronchial pneumonia, in addition to the presence of intra-alveolar fibrin, erythrocytes, neutrophils, and foci of macrophages, focal abscess formation, thickened alveolar septa, and pneumocyte hyperplasia. Despite the use of special histochemical staining, including Gomori methenamine silver, acid-fast stain, Gram stain, and immunohistochemical staining for cytomegalovirus, herpes simplex virus, and adenovirus, no microorganisms were identified at that time. Tissue blocks (formalin fixed, paraffin embedded) from the lung biopsy specimen were retrieved. DNA was extracted from the tissue, broad-range 16S rRNA.
gene primers were used for PCR and sequencing (1), and L. anisa was detected in the lung biopsy specimen with a 100% sequence match. (It should be noted that we have performed many 16S rRNA gene PCR assays with other, unrelated, formalin-fixed and paraffin embedded tissue, but Legionella species has never been detected; cross contamination is not a concern.) Since it had been reported that Legionella species bacteria are well stained by Giemsa stain (2), Giemsa staining was performed and it highlighted the intracellular organisms. The organisms were more coccobacillus like, rather than slender long bacilli, consistent with the reported morphology of intracellular Legionella species (Fig. 2) (3). No extracellular organisms were detected in the lung section.

**Discussion.** Legionella species are widely present in nature, mostly in soil and water. They are intracellular organisms. In freshwater, they are parasites of protozoa and they may also multiply in mammalian cells. These organisms may cause pneumonia when a susceptible host inhales aerosols containing the bacteria. L. pneumophila is responsible for most of the reported cases of Legionnaires’ disease in the United States (4). L. anisa has been associated with the less severe form of legionellosis Pontiac fever (11) and, much less frequently, pneumonia (5) and pleural infection (6) in immunocompromised patients. L. anisa, in addition to L. pneumophila, is reported to be the major species implicated in hospital water system contamination (7). Extrapulmonary infections due to Legionella spp. are rare but may occur in immunocompromised patients. They may occur as primary infections or because of dissemination from another source, most commonly the lung.

Osteomyelitis of the patella is uncommon and is generally considered a disorder of childhood, with most cases occurring between 5 and 10 years of age (8). Staphylococcus aureus is the organism most commonly responsible. Other reported causes include Streptococcus species, Mycobacterium intracellulare, Escherichia coli, Clostridium bifermentans, syphilis, and mycoses (9). Factors that predispose to patellar osteomyelitis include direct trauma, septic prepatellar bursitis, and septic arthritis. The clinical presentation is varied, ranging from acute with systemic signs of infection to insidious with only mild localized

FIG 1 Gram staining (with safranin as a counterstain) of patella tissue showing a cell with intracellular (A) and extracellular (B) Gram-negative rods.

FIG 2 Giemsa staining of a lung tissue biopsy specimen showing intracellular organisms.
symptoms (3). We believe that in our case L. anisa was the true pathogen, given the radiologic and operative findings consistent with infection involving the patella, in addition to direct identification of the organism by 16S rRNA gene sequencing and positive culture from the biopsy specimen.

Our patient had been hospitalized 2 months prior to the onset of knee pain for multifocal pneumonia, with no infectious etiology identified in bronchoalveolar lavage or tissue samples, despite the use of special stains and cultures for bacteria, fungi, acid-fast bacilli, and viruses. However, samples had not been cultured on BCYE agar and the patient’s antibiotic management included azithromycin. Therefore, the close temporal relationship of the episode of pneumonia and subsequent patellar infection raised the suspicion that L. anisa was the primary causative pathogen in the earlier pulmonary infection. Indeed, through the use of 16S rRNA gene sequencing, L. anisa was identified in the formalin-fixed, paraffin embedded tissue blocks from the lung biopsy specimen, leading to the conclusion that his pneumonia had been the source of the L. anisa bacteria implicated in his patellar osteomyelitis.

Osteomyelitis due to Legionella spp. has been reported only once in the literature. The patient was a middle-aged woman with systemic lupus erythematosus who was receiving corticosteroids and was diagnosed with concomitant pneumonia and osteomyelitis of the tibia due to Legionella longbeachae (10). Other extrapulmonary infections involving Legionella spp. are suspected to arise from hematogenous dissemination and tend to occur in immunocompromised patients.

REFERENCES