**Legionella anisa** Osteomyelitis of patella: A Case Report

'Martha Cristina Sanchez**2**, Rani Sebti2, Patrice Hassoun3, Ciaran Mannion3, Andre H Goy4, Tatyana Feldman4, Anthony Mato4, Tao Hong1*

Microbiology and Molecular Pathology1, Surgical Pathology3, Department of Pathology, Division of Infectious Diseases2, John Theurer Cancer Center4, Hackensack University Medical Center, Hackensack NJ,

**Running title:** *Legionella anisa* Osteomyelitis

**Keywords:** *Legionella anisa*, Osteomyelitis, Patella, 16S rDNA gene, Nucleotide sequence

* Corresponding author: Dr. Tao Hong

Department of Pathology, Clinical Microbiology & Molecular Pathology Laboratory, Hackensack University Medical Center, 30 Prospect Ave, Hackensack, NJ 07601

Tele: 201-996-4854. Fax: 201-996-2156. E-mail: thong@humed.com

**Current address:**

Martha Cristina Sanchez

Greater New Bedford Community Health Center

Infectious Disease Department,

874 purchase street, New Bedford, MA 02740
Abstract

A 51-year-old man with history of stage IV angioimmunoblastic T-cell lymphoma was diagnosed with osteomyelitis of the patella. *Legionella anisa* was identified by 16S rDNA sequencing and culture. The patient had pneumonia two months prior to this osteomyelitis episode. *L. anisa* was retrospectively detected from the lung tissue by 16S rDNA sequencing and was considered the source of the *L. anisa* for the 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 three-week history of right knee pain. The pain was associated with 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 left-sided tonsillectomy and was treated with Alemtuzumab (anti CD52 monoclonal antibody) and Lenalidomide. Subsequently, two months prior to his current admission, he was also hospitalized with multifocal pneumonia and underwent a bronchoscopy with transbronchial lung biopsy. The latter showed bronchiolitis obliterans with organizing pneumonia (BOOP) 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 1 gram every 12 hours, ceftazidime 2 grams IV every 8 hours, azithromycin 500 mg IV every 24 hours and voriconazole, 200 mg PO every 12 hours. He recovered clinically with resolution of pulmonary infiltrates on computerized axial tomography (CAT) 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 (MRI) 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 stain of the tissue demonstrated numerous white blood
cells, red blood cells and large numbers of gram-negative rods. He was initially treated
with imipenem/cilastin 500 mg IV every 6 hours. After *L. anisa* was identified (see
microbiology investigation), the patient was treated for 8 weeks with moxifloxacin 400
mg IV daily and had an uneventful course.

**Microbiology investigation**

A bone (patellar) biopsy specimen showed numerous extracellular and intracellular
slender gram-negative rods (Figure I). Routine aerobic and anaerobic cultures were
negative. To rapidly identify the pathogen, DNA was directly extracted from the biopsy
specimen using the MagneSiI Genomic, Fix Tissue System (Promega, WI). Broad range
polymerase chain reaction (PCR) primers, capable of amplifying the first 5-525 base
pairs of the 16s rRNA gene (8), were used to amplify the DNA and the nucleotide
sequence of the amplicon was determined. Compared with the data base of Genbank, a
100% match was obtained with *L. anisa*. After successful direct identification of the *L.
anisa* nucleic acid from the biopsy specimen, culture was undertaken using BCYE agar
(BBL, Becton and Dickinson, Sparks, Maryland, USA). Following incubation at 35°C,
5% CO₂, a pure culture of numerous white colonies appeared after 3 to 4 days. Gram
stain (with safranin as counter stain) revealed slender gram-negative rods. Under long
wave UV light, the colonies exhibited bright white-blue fluorescence. We have
confirmed that the isolate only grows on BCYE agar, but not on 5% SBA. 16S rDNA
sequencing was performed on the bacterial colony and a 100% DNA sequencing match was obtained with *L. anisa*.

In the context of the identification of *L. anisa* from the patella, the patient’s recent presentation with pneumonia was re-assessed. The pathology report had noted acute bronchial pneumonia, in addition to the presence of intra-alveolar fibrin, erythrocytes, neutrophils and focally macrophages, focal abscess formation, thickened alveolar septa and pneumocyte hyperplasia. Despite the use of special histochemical stain, including Gomori Methenamine Silver (GMS), acid-fast stain, Gram Stain, and immunohistochemical stain for cytomegalovirus (CMV), herpes simplex virus (HSV) and adenovirus, no microorganisms had been identified at that time. Tissue blocks (formalin fixed, paraffin embedded) from the lung biopsy were retrieved. DNA was extracted from the tissue and broad range 16S rDNA gene primers were used for PCR and sequencing (8), *L. anisa* was detected from the lung biopsy specimen with 100% sequence match (It should be noted that we have performed many 16s rDNA PCR using 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 *Legionella* species is well stained by Giemsa stain (4), a Giemsa stain was performed and highlighted the intracellular organisms. The organisms were more coccobacilli-like, rather than slender long bacilli, consistent with the reported morphology of intracellular *Legionella* species (Figure II) (3). No extracellular organisms were detected in the lung section.
Legionella species are widely present in nature, mostly in soil and water. They are intracellular organisms. In fresh water, 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 diseases in the United State (6). *L. anisa* has been associated with the less severe form of *legionellosis*-Pontiac fever- and, much less frequently, pneumonia (7) and pleural infection (1) in immunocompromised patients. *L. anisa*, in addition to *L. pneumophila*, is reported to be the major species implicated in contamination of hospital water system (12). Extrapulmonary infections due to *Legionella spp.* are rare, but may be seen in immunocompromised patients. They may occur as a primary infection or due to 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 (11). *Staphylococcus aureus* is the most common organism. Other reported pathogens include the following: *Streptococcus species, Mycobacterium intracellulare, E. coli, Clostridium bifermentans*, syphilis and mycoses (2). Predisposing factors to patellar osteomyelitis include direct trauma, septic prepatellar bursitis, and septic arthritis. Clinical presentation is varied, ranging from acute with systemic signs of infection to insidious with only mild, localized symptoms (3). We believe in our case *Legionella 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 rDNA sequencing and positive culture from the biopsy specimen.

Our patient had been hospitalized two months prior to the onset of knee pain for multifocal pneumonia, with no infectious etiology identified on samples from bronchoalveolar lavage or in tissue, despite 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 suspicion that \textit{L. anisa} might have been the primary causative pathogen in the earlier pulmonary infection. Indeed, through the use of 16S rRNA gene sequencing, \textit{L. anisa} was identified in the formalin fixed, paraffin embedded tissue blocks from the lung biopsy, leading to the conclusion that the pneumonia had been the source of \textit{L. anisa} implicated in the patellar osteomyelitis.

Osteomyelitis due to \textit{Legionella spp.} has only been reported 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 \textit{Legionella longbeachae} (9). Other extra pulmonary infections involving \textit{Legionella spp.} are suspected to arise from hematogenous dissemination and tend to occur in immunocompromised patients.
References


Figure I legend.

Gram stain (with safranin as counter stain) of patella tissue shows cell with intracellular (a) and extracellular (b) Gram negative rods.
Figure II
Figure II legend

Giemsa stain of lung biopsy tissue shows intracellular organisms.