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Mistaken identity: *Legionella micdadei* appearing as acid fast bacilli on lung biopsy of a hematopoietic stem cell transplant patient

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Abstract

Legionella micdadei is a potential cause of invasive lung infections in immunocompromised hosts. On biopsy specimens, it can appear as an acid-fast bacillus (AFB) and can be mistaken for a member of genus *Mycobacterium*. As *Legionella* requires selective media to grow in culture, and a commonly used, commercially available urine antigen test for *Legionella* only detects *Legionella pneumophila* serogroup 1 but not *L. micdadei*, it is important to consider this organism in the differential diagnosis for AFB in immunocompromised hosts. We report a case of *Legionella micdadei* infection, which was initially treated empirically for non-tuberculous mycobacteria based on acid fast staining of biopsy tissue before the final diagnosis was made.

Keywords

Legionella micdadei; Legionellosis; Pneumonia; Hematopoietic stem cell transplant

Unique among *Legionella* species, *Legionella micdadei* can appear as an acid-fast bacillus when stained in tissue specimens. Here we report a case where *Legionella micdadei* was misidentified as a *Mycobacterium* species upon transcutaneous biopsy of a consolidative lung lesion in a patient after allogeneic hematopoietic stem cell transplantation (HSCT).

A 68 year old man with a history of familial Kaposi's sarcoma (KS) and precursor T cell acute lymphoblastic leukemia with concurrent myelodysplastic syndrome underwent reduced intensity chemotherapy conditioning followed by an unrelated HSCT. His preparative regimen included fludarabine, BCNU, melphalan and thymoglobulin. The patient engrafted on post-transplant day (PTD) +15, but his post-transplant course was complicated by acute graft-versus-host disease (GVHD) to the skin, for which he was maintained on high dose corticosteroids, tacrolimus and mycophenolate. On PTD +23, the patient was also started on posaconazole as antifungal prophylaxis, but it was discontinued 8 days later due to elevation of his liver enzymes. On PTD +48, the patient presented with fever, fatigue and a non-productive cough.

On physical examination, his temperature was 38.1°C and he was mildly tachycardic, with a pulse of 94. Otherwise, his vital signs were stable and he was not in any acute distress. The lung exam was remarkable for focal dullness over his left lower lung field. He was also noted to have a diffuse morbilliform rash, thought consistent with GVHD. Chest radiography revealed a left lower lobe infiltrate, which was confirmed by CT Thorax (Figure 1). Laboratory data were notable for: white blood cell 10,500 cells/dL, hemoglobin 12.2 gm/dL, platelets 111 cells/dL, creatinine 1.0 mg/dL, total bilirubin 3.2 mg/dL, AST 328 U/L and ALT 795 U/L. Initial workup also included two sets of peripheral blood cultures, aspergillus galactomannan (serum), histoplasma antigen (serum and urine), cryptococcal antigen (serum), as well as nasopharyngeal respiratory virus screen by direct immunofluorescence, which were all negative. Given the concern of fungal versus bacterial pneumonia, the patient was started empirically on liposomal amphotericin B and levofloxacin. On PTD +53, a CT-guided fine needle aspiration biopsy of the left lung lesion was undertaken. Gram Stain, Hematoxylin & eosin, Grocott-Gomori's methenamine silver and periodic acid-Schiff stains were negative for micro-organisms against a background of necrotic tissue with obliterated lung architecture. However, Kinyoun and Fite stains revealed the presence of AFB (Figure 2).

Given the finding of AFB by staining, the patient was started on empirical treatment for non-tuberculous mycobacteria, since the patient did not have any known risk factors for tuberculosis. Initial bacterial, fungal and AFB cultures from biopsy did not yield any growth. In attempt to identify the AFB, broad range PCR of the bacterial 16S ribosomal subunit RNA was performed on the formalin-fixed paraffin embedded tissue after deparaffinization. Amplification was successful with the resulting sequence 100% homologous with reference sequences for *L. micdadei*. The lung biopsy tissue samples were then re-cultured on buffered charcoal yeast extract (BCYE) supplemented with iron, cysteine, α -ketoglutarate, polymixin B, anisomycin, and vancomycin, and successfully grew colonies of *L. micdadei* on day 3 (Figure 3), confirming the molecular diagnosis.

L. micdadei (also known as *Tatlockia micdadei* and *L. pittsburghensis*) was first described as a human lung pathogen in 1979 when Pasculle and colleagues (1) in Pittsburgh isolated a Gram-negative, weakly acid-fast bacillus from the lung tissues of two renal transplant recipients with acute purulent pneumonia, and the organism was designated "Pittsburgh pneumonia agent" (PPA). Later that year, PPA was identified as the causative agent of pneumonia in a total of 8 immunosuppressed patients in Pittsburgh (2) as well as in another 5 immunosuppressed patients in Virginia (3). The isolation of this bacilli led to the retrospective identification of two rickettsia-like organisms TATLOCK and HEBA that were first encountered in 1943 and 1959, respectively (4). The organism was formally named *L. micdadei* in 1980, in honor of Joseph E. McDade, who first isolated *L. pneumophila* (5).

Legionella species are fastidious, facultative intracellular Gram-negative bacilli that fail to grow on many standard media routinely used in Clinical Microbiology, but can be isolated on BCYE agar (5). Unique among *Legionella* species, *L. micdadei* is weakly acid-fast in clinical specimens and when cultivated in embryonated eggs, but it loses this characteristic when grown in culture. Due to its weakly acid-fast characteristic, the organism can be

mistaken as a mycobacterium and not infrequently, patients might receive treatment for presumed mycobacterial infection (3, 6, 7), as in our case.

Diagnosis of this bacteria on tissue specimens is further complicated by poor uptake of counterstain by *Legionella micdadei* with standard gram staining, which often makes initial identification of this gram-negative organism difficult(8–12). In this case, there was both a relative paucity of organisms and weak uptake of counterstain which lead to an initial negative read of the gram stain. Repeat post-hoc examination of the initial gram stain after the diagnosis was confirmed did reveal rare, very weakly counterstained gram-negative rods consistent with legionella, but difficult to distinguish from the necrotic background. Alternative staining methods, such as Wright-Giemsa or “Diff-quick” may also be effective for detecting legionella on cytologic preparation, but was negative in this case(8). Impregnated silver staining techniques such as Deiterle, Warthin-Starry, and Steiner methods may be sensitive for *Legionella*, but can be complicated by high amounts of background staining, and were not performed in this case(8, 9, 12, 13). The Grocott-Gomori’s methenamine silver stain used in this case is not sensitive for bacteria and was used to screen for the presence of fungi(8, 9). Histology of *Legionella spp* infections is classically consistent with an acute fibrinopurulent bronchopneumonia(12). This case may have been more unusual histologically given that there was a high degree of necrosis and obliterated lung architecture.

L. micdadei is the second most common cause of legionellosis, accounting for 6–8% of cases in the US (14, 15). Similar to *L. pneumophila* and other *Legionella spp.*, *L. micdadei* usually causes infection of the respiratory tract, most commonly manifested as lobar pneumonia, while lung abscesses or lung nodules have also been described (16–18). Few extrapulmonary cases have been reported, including cutaneous infection (19, 20), brain abscess (21), prosthetic valve endocarditis (22) and prosthetic joint infection (23). Most cases of *L. micdadei* infection, including a nosocomial outbreak (24), involved immunocompromised patients. Patients on high dose corticosteroid therapy, or recipients of solid-organ transplantation represent the majority (2, 3); those with malignancies, HSCT and HIV/AIDS (7, 25, 26) are also at risk. Rarely, immunocompetent hosts can also succumb to *L. micdadei* pneumonia (27, 28), although many were found to have other comorbidities (29, 30). Clinical and radiographic features of *L. micdadei* pneumonia are indistinguishable from those caused by other *Legionella spp.* (14, 29, 30). Immunocompromised hosts often present with high fevers and nonproductive cough (as in our patient); pleuritic chest pain is also a common feature. Contrarily, productive cough and low-grade fever are more common among immunocompetent patients, and pleuritic chest pain is rare (14). In one study, other non-specific symptoms or signs associated with *L. micdadei* pneumonia include changes in mental status (60%), abdominal pain (36%) and diarrhea (26%) (30). While mild diarrhea is common with *Legionella* infection, massive diarrhea has been noted in some cases (17, 31). For treatment of *L. micdadei*, erythromycin was the main stay of treatment historically, but the newer macrolides or fluoroquinolones are now considered the therapy of choice (32). Of interest, unlike *L. pneumophila*, *L. micdadei* does not produce beta-lactamase and is susceptible to penicillins and cephalosporins in vitro (33). However, there is little correlation between in vitro susceptibility testing and clinical efficacy for *Legionella spp.*

For laboratory diagnosis, *Legionella* urine antigen only detects *L. pneumophila* serogroup 1, thus not useful for the detection of *L. micdadei* or other *Legionella* spp. Definitive diagnosis of *L. micdadei* infection relies on the isolation of the organism on selective media. Direct fluorescent antibody staining on clinical specimens and antibody seroconversion are also employed for diagnosis, but the sensitivity and/or specificity are uncertain, and likely low (14). More recently, molecular amplification and detection (e.g. with 16S rRNA PCR) have been used successfully to identify *L. micdadei* as the pathogen in a few cases, including this report (21–23), but this test is not commercially available. Clinicians should be alerted to the possibility of *L. micdadei* infection in susceptible hosts and should ensure that lung biopsy specimens in patients with undiagnosed pneumonia are sent for culture with *Legionella* specific media. They should continue to keep this organism on their differentials if AFB is observed in clinical specimens by staining.

For our patient, after the AFB was identified as *L. micdadei*, he had initial clinical response while on levofloxacin as well as radiologic improvement per repeat CT Thorax on PTD + 77. However, he further developed worsening GVHD which necessitated an increase of immunosuppression, as well as a recrudescence of his KS with skin and visceral involvement. Given a decline of his clinical status, a pan-body CT was obtained on PTD + 98, which showed enlargement of the left lower lobe consolidation. On PTD + 105, Levofloxacin was changed to Azithromycin after 52 days of continuous quinolone therapy and CT-guided transthoracic biopsy was repeated. This specimen again grew *L. micdadei* in culture on supplemented BCYE agar. Given the possibility of nosocomial acquisition of this pathogen, hospital infection control was notified and an investigation was conducted. Taps and showerheads in hospital patient rooms that the patient had access to prior to diagnosis were cultured and found negative for pathogens, including *Legionella* spp. No other cases of this pathogen were detected in the hospital during this time period. The patient was thought to have acquired *L. Micdadei* in the community where he was staying in the 3 weeks prior to onset of infection, however this could not be confirmed by culture. The patient eventually succumbed to complications from GVHD and disseminated KS. He died on PTD +157.

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Figure 1.
CT Thorax demonstrating a peripheral left base consolidation with air bronchogram.

Figure 2. CT-guided fine needle aspiration biopsy of the left lung lesion showing necrotizing inflammation with acid fast organisms (Kinyoun (AFB) stain).

Figure 3.
BYCE plate showing colonies of *L. micadadei* from the patient's lung biopsy specimen.