

Septic Arthritis Caused by *Legionella dumoffii* in a Patient with Systemic Lupus Erythematosus-Like Disease[∇]

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We describe a patient with systemic lupus erythematosus (SLE)-like disease on immunosuppressive treatment who developed septic arthritis of the knee involving *Legionella dumoffii*. Cultures initially remained negative. A broad-range 16S PCR using synovial fluid revealed *L. dumoffii* rRNA genes, a finding that was subsequently confirmed by positive *Legionella* culture results.

CASE REPORT

A 58-year-old female patient with systemic lupus erythematosus (SLE)-like disease was admitted to the Rheumatology Department of Sint Maartenskliniek Nijmegen, Nijmegen, Netherlands, in early May 2008 for treatment of septic arthritis of the right knee. She was known since 2002 to have had symmetrical peripheral polyarthritis, nailfold lesions, and antibodies against double-stranded DNA and cardiolipin (symptoms classified as representing incomplete SLE or an SLE-like disease). Concurrent immunosuppressive therapy consisted of administration of methotrexate (7.5 mg) weekly and hydroxychloroquine (400 mg) and prednisolone (10 mg) daily. In the week prior to admission, the patient had received an intra-articular injection of triamcinolone acetonide for mild chronic arthritis of the right knee. Two days later, she developed a warm, tender, and swollen right knee. The patient had a leukocytosis count of 15.2×10^9 /liter and a slightly increased erythrocyte sedimentation rate (ESR) (20 mm/h) and C-reactive protein level (22 mg/liter). Twenty milliliters of purulent aspirate (white blood cell [WBC] count, 78×10^9 /liter) was obtained. Results for Gram staining (fuchsin counterstain) on joint aspirate were negative, and results for cultures grown on standard and mycobacterial media also remained negative. Polarized light microscopy did not reveal any crystals in the joint aspirate. A chest X-ray showed no abnormalities. The tuberculin skin test was negative.

The patient was started on flucloxacillin (6 gr/day) and ciprofloxacin (400 mg twice daily) intravenously; treatment was switched to oral therapy after 9 days, after clinical improvement of the arthritis. The total duration of antibiotic therapy was 6 weeks.

One month later, she was readmitted under suspicion of recurrent septic arthritis of the right knee. On admission, she also had arthritis of two metacarpophalangeal (MCP) joints of

the right hand, a purulent blister on the palmar side of the right thumb, a new systolic heart murmur, and a fever spike (39.1°C [102.4°F]). Biochemistry showed an increased ESR (58 mm/h) and C-reactive protein level (160 mg/liter), as well as a slightly elevated WBC count (10.5×10^9 /liter).

With effort, 1 ml of purulent material was aspirated from the knee joint; the aspirated material was found to contain an uncountable but high level of leukocytes. Purulent aspirate was also obtained from the blister. Blood cultures grown on standard media remained negative. Samples of repeated joint aspirates and the blister aspirate were inoculated onto standard and mycobacterial culture media, including direct inoculation of joint aspirate into mycobacterial blood culture vials (Bactec 13A TB media). Transesophageal echocardiography identified two round, nonmobile structures 3 mm in diameter on the aortic valve.

All cultures remained sterile for 12 days, and a 16S broad-range PCR was additionally performed on joint aspirate material after consultation with the medical microbiologist. In order to avoid false-positive PCR results, the various steps of the PCR procedure (DNA isolation, pre- and post-PCR handlings) were performed in dedicated separate facilities. DNA isolation was done using the method described by Boom et al. (3) with minor modifications. An approximately 500-nucleotide (nt) portion from the 5' end of the 16S rRNA gene was amplified using broad-range primers (5'-CCTAACACATGC AAGTCGARGC-3' and 5'-CGTATTACCGCGGCTGCT-3') under standard conditions. Negative controls were included that also underwent the DNA extraction procedure. All PCRs were performed in duplicate. Amplification reactions spiked with a small amount of control DNA showed that the purified DNA samples were free from PCR inhibitors. After 35 cycles of amplification, a clear PCR product was visible on agarose gels in both duplicate amplification reactions of the clinical sample whereas the negative controls showed no amplification products. The obtained PCR product was purified using SPRI chemistry (Beckman Coulter, Mijdrecht, Netherlands) and sequenced on a MegaBACE 500 automated DNA analysis platform (GE Healthcare, Diegem, Belgium) using a DYEnamic dye terminator kit (GE Healthcare) as recommended by the manufacturer. The obtained sequence was compared to the entries of the GenBank public database using the BLAST

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interface (NCBI BLAST [Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/>]) (14). The obtained sequence was a 100% match to those of *Legionella dumoffii*. Joint aspirates collected on two separate days were subsequently inoculated on a buffered charcoal yeast extract (BCYE) culture plate, which was incubated at 37°C (98.6°F) under humidified conditions. Only one BCYE agar plate was used for isolation of *Legionella* species, consisting of a *Legionella* CYE agar base (Oxoid, Basingstoke, The United Kingdom) supplemented with *Legionella* BCYE growth supplement (Oxoid, Basingstoke, The United Kingdom) and *Legionella* MWY selective supplement (Oxoid, Basingstoke, The United Kingdom). Both cultures grew around 15 to 100 colonies after 2 days. DNA sequence analysis of the grown culture yielded the same sequence as obtained earlier using DNA extracted from the clinical samples.

Treatment with ciprofloxacin (400 mg) three times a day intravenously and oral rifampin (600 mg) twice daily was started after obtaining the second specimen. The hand arthritis improved quickly, but the arthritis of the right knee persisted until surgical joint drainage was performed to improve recovery. The patient was discharged 5 weeks after admission.

Antibiotic treatment was continued for a total duration of 3 months. One year after admission, no signs of arthritis were present. A conventional radiograph of the knee showed significant medial and lateral joint space narrowing, indicating substantial cartilage loss. Echocardiographical reevaluation after 6 months showed unchanged aorta valve lesions, but no aortic insufficiency or other signs of heart failure were present.

This report describes a case of septic arthritis caused by *L. dumoffii* in an immunocompromised patient. It is considered interesting for several aspects. The pathogen *L. dumoffii* has not been reported previously in cases of septic arthritis. Other *Legionella* species, especially non-*L. pneumophila* spp., seldom cause septic arthritis (1, 2, 5, 9, 11). Furthermore, the pathogen was not identified by standard bacterial cultures and was first identified only by applying 16S broad-range PCR. Empirical antibiotic therapy initiated according to national guidelines should have been adequate but was not, indicating that when a first septic arthritis episode is caused by *L. dumoffii*, the standard treatment period may need extension in those cases. Eventually, longstanding antibiotics treatment resulted in complete resolution of the infection, but substantial loss of joint cartilage had already occurred.

Infections due to *Legionella* species mostly present as Legionnaires' disease (caused by *L. pneumonia*) and, to a lesser extent, as Pontiac fever, a self-remitting flu-like illness (4, 10). *Legionella* pneumonia can be accompanied by severe systemic illness. Localized extrapulmonary infection is very rare. It may occur during or following *Legionella* pneumonia, presumably resulting from hematogenous spread (10). Wound, soft tissue, or surgical infections may also occur after direct inoculation of the skin. Occasionally, no clear route of infection can be established and signs of concurrent or preceding pneumonia are absent. Sites of extrapulmonary infection include the heart (prosthetic heart valves, myocardium, pericardium), soft tissue (skin, muscle), lymph nodes, and internal organs (spleen, liver,

kidney) (8, 10). The majority of extrapulmonary *Legionella* infections occur in immunocompromised hosts (10).

Legionellae species are fastidious Gram-negative aerobic bacteria. Over 90% of all *Legionella* pneumonia and extrapulmonary legionellosis cases are caused by *L. pneumophila* (4). *L. dumoffii* is one of 20 non-*L. pneumophila* species that have been reported to be pathogenic in humans on the basis of their isolation from clinical specimens (4). *L. dumoffii* has been identified in 0.2% to 0.6% of all culture-positive pneumonia cases in survey studies (12, 13). Reports of extrapulmonary infection with *L. dumoffii* are sparse, consisting of a case of pericarditis and a small cluster of prosthetic valve endocarditis (10).

Reports of joint infections by *Legionellae* species are extremely rare. Six cases of arthritis caused by *Legionella* bacteria, including the present case, have been described (1, 2, 5, 9, 11) and are summarized in Table 1. Interestingly, oligoarthritis, affecting two or more joints, was described for five patients. In four cases, culture results were positive. Three patients were reported as not having had a preceding or concurring case of pneumonia. In two patients, arthritis was reported to be destructive. In one case (case 2 in Table 1), the arthritis was considered to be reactive instead of septic in nature, as pneumonia had occurred 6 months before; the WBC count in synovial fluid was very low and culture results were negative (1).

The patient in the present case was immunocompromised because of her underlying SLE-like disease, which was treated with methotrexate and prednisolone. The route of infection was not identified. A low-grade bacterial infection is hypothesized, exacerbating the severity of the condition after an intra-articular injection with triamcinolone acetate (single-use vial). Libman-Sachs endocarditis complicating the patient's SLE-like disease is the most likely explanation for the aortic valve abnormalities. *L. dumoffii* as a contributing factor was very unlikely, based on the absence of Duke criteria for the diagnosis of infective endocarditis and on the lack of validated reports of native valve endocarditis caused by any *Legionella* spp.

Diagnosis of *Legionella* infections is complicated. *Legionellae* species do not grow on standard media. Special media (BYCE) and adequate processing are needed for optimal isolation (4). Isolation from joint aspirates after the use of mycobacterial media and chocolate agar media has been reported anecdotally (see Table 1) (2, 9). The urinary antigen test is sensitive only for identification of infections with *L. pneumophila* serogroup 1. Antibody detection by enzyme-linked immunosorbent assays (ELISA) may lack sensitivity and specificity, and conversion to seropositivity may take several weeks. In isolated extrapulmonary infections, underreporting may be an even greater issue because of the absence of pulmonary symptoms.

Improved diagnostic methods are needed, and 16S broad-range PCR may be a valuable diagnostic aid. In cases of culture-negative septic arthritis, 16S broad-range PCR techniques can aid in identifying the causative microorganism. The technique identifies 16S ribosomal DNA sequences that are species-specific for many microorganisms (6). The fast result, obtained within 36 to 72 h, is a major advantage, especially for septic arthritis, which often results in extensive joint destruction in cases of delayed treatment initiation. Besides culture-negative infections, PCR can also aid in pathogen identifica-

TABLE 1. Arthritis caused by *Legionella* species^a

Case	Reference or source	Gender	Age (yr)	Medical history (immunosuppressive therapy)	Clinical diagnosis	Species	Diagnostic methods	WBC ($\times 10^9$ /ml)	Remarks
1	1	Male	32	None	Reactive arthritis, right knee and ankle	<i>L. pneumophila</i> (unspecified strain)	Positive serology and UAT	0.8	Large hematoma, right calf
2	2	Male	51	Thymoma (yes)	Septic arthritis, left knee and right ankle	<i>L. pneumophila</i> SG 1	Positive serology (IgG, IgM), UAT, and culture (aspirate and blood)	40	<i>Legionella</i> species grew from mycobacterial medium
3	5	Male	56	RA (yes)	Septic arthritis, left TMT4 and -5, left wrist, right DIP4	<i>L. longbeachae</i>	Positive Gram staining and culture (aspirate)	n.m.	Neutropenia
4	9	Female	80	Osteoarthritis (no)	Septic arthritis, MCPs and PIPs, right hand	<i>L. pneumophila</i> SG 4	Positive tissue culture and IgG antibodies	n.m.	Brownish synovium; <i>Legionella</i> species grew from chocolate agar plate
5	11	Female	80	Chronic kidney disease (no)	Septic arthritis, left ankle	<i>L. pneumophila</i> SG 1	Positive UAT and 16S-PCR	50	Onset of arthritis after 16 days of antibiotics
6	Present case	Female	58	SLE-like disease (yes)	Septic arthritis, left knee and right MCP2 and -3	<i>L. dumoffii</i>	Positive culture and 16S-PCR (aspirate)	Uncountable	Relapse with purulent blister; cardiac valve abnormalities

^a WBC, white blood cell count in joint aspirate; DIP, distal interphalangeal joint; MCP, metacarpophalangeal joint; n.m., not mentioned; PIP, proximal interphalangeal joint; RA, rheumatoid arthritis; SG, serogroup; SLE, systemic lupus erythematosus; TMT, tarsometatarsal joint; UAT, urinary antigen test.

tion in situations where antibiotics already have been initiated or in cases with polymicrobial culture results. Disadvantages include the possibility of false-positive results. Positive 16S PCR results have been reported more often (at a differential of up to 20%), in cases of patients who have underlying inflammatory joint diseases, as rheumatoid arthritis and reactive arthritis (6, 7). The reasons for these results are speculative. Bacterial DNA may be retained for longer periods of time in joints which are chronically inflamed. Another explanation is that the risk for contamination may be higher in specimens from chronically inflamed joints, as they may contain a higher level of endogenous free DNA serving as carrier DNA for bacterial DNA traces picked up during the PCR procedures. It warrants the use of many controls in each PCR procedure to ensure correct interpretation of the data.

In conclusion, we describe a very unusual pathogen, *L. dumoffii*, as a cause of septic arthritis in a clinical emergency with significant morbidity and mortality. The case illustrates that the absence of signs and symptoms of pulmonary involvement does not exclude a *Legionella* infection. It further underlines the possibility of applying 16S broad-range PCR as a novel solution to the diagnostic problems encountered in cases of culture-negative septic arthritis.

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