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# *Mycoplasma pneumoniae* Periprosthetic Joint Infection Identified by 16S Ribosomal RNA Gene Amplification and Sequencing

## A Case Report

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*Investigation performed at Washington University School of Medicine, St. Louis, Missouri*

Chronic, culture-negative septic arthritis represents a major clinical dilemma. It often results in prolonged courses of antimicrobial therapy, which may or may not have activity against the organism responsible for the infection. It is not possible to cultivate all types of pathogens *in vitro* with use of traditional laboratory testing methods, but emerging techniques can be helpful to define the etiology of these infections in some cases. We describe a case of a periprosthetic joint infection in a thirty-year-old woman with juvenile rheumatoid arthritis (JRA) and hypogammaglobulinemia, who developed septic arthritis after prolonged pneumonia. She received multiple courses of antimicrobial therapy without improvement of the joint symptoms. The microbiologic diagnosis was made with use of 16S ribosomal RNA (rRNA) gene amplification and sequencing from synovial fluid and tissue. The patient gave permission for this information to be submitted for publication.

### Case Report

A thirty-year-old woman with JRA and hypogammaglobulinemia was referred for evaluation of an infection at the site of a total knee arthroplasty. Her JRA had been managed with multiple courses of corticosteroids, infliximab, and, more recently, rituximab. She had undergone bilateral shoulder and knee synovectomy, and right elbow synovectomy, and finally had required left total knee arthroplasty in 2006.

In the fall of 2009, the patient was hospitalized because of pneumonia, which had a prolonged course, with several hospitalizations due to recurrences over two months. Chest radiographs confirmed right upper lobe pneumonia, which later progressed to bilateral pneumonia. She was treated with azithromycin and ceftriaxone and initially showed improvement, but she then had a relapse with recurrent symptoms. During

the multiple relapses, she was treated with vancomycin and cefepime followed by ertapenem. She had five bronchoscopy procedures. The bronchoscopies all showed purulent secretions, but all cultures were negative, including fungal and mycobacterial cultures. Polymerase chain reaction (PCR) for *Mycoplasma pneumoniae* from a nasopharyngeal swab was also negative. Her condition improved in October 2009, and the course of antibiotics was discontinued.

In November 2009, the patient developed redness, pain, and swelling at the site of the prosthetic left knee. Arthrocentesis revealed 50,500 nucleated white blood cells in the synovial fluid, 79% of which were neutrophils. She underwent irrigation and drainage with synovectomy of the knee, but the prosthesis was retained. Copious amounts of yellow turbid fluid were found intraoperatively, but all cultures were negative. The patient received six weeks of empiric antibiotic treatment with intravenous (IV) vancomycin and oral ciprofloxacin and had clinical improvement. She was then started on chronic suppressive therapy with oral trimethoprim-sulfamethoxazole.

At a clinic visit several weeks later, she was noted to have persistent knee redness and swelling while she was still taking trimethoprim-sulfamethoxazole. She was then admitted for evaluation of presumed recurrent periprosthetic joint infection. Arthrocentesis at this time revealed 48,000 nucleated white blood cells in the synovial fluid, 89% of which were neutrophils. In January 2010, the patient had repeat surgical drainage and irrigation with removal of the total knee implant as well as spacer placement. During this procedure, two batches of cement were used for the spacer block and a third batch of cement was used for the intramedullary dowels, with 2 g of vancomycin and 2.4 g of tobramycin used in each batch of cement. All intraoperative cultures were negative. Empiric

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antibiotic treatment, with daptomycin, ciprofloxacin, and metronidazole, was resumed.

In December 2009, the patient had had a low immunoglobulin G (IgG) level, low IgA level, and suppression of all IgG subclasses, along with a very poor response to a test dose of pneumococcal vaccine. These changes were thought to be due to her prior treatment with rituximab. IV immunoglobulin infusions were started during the January 2010 hospitalization. Because of the recurrent knee joint infection and negative cultures, samples of synovial tissue and fluid were sent for 16S rRNA gene amplification, and sequencing was obtained for the samples of synovial tissue and fluid. Both samples were strongly positive for *M. pneumoniae*. An organism-specific PCR for *M. pneumoniae* (targeting the P1 gene) was performed on the knee tissue and was also positive. Bronchoalveolar lavage fluid obtained during her hospitalization with pneumonia in September 2009 was retrospectively assayed by PCR and was found to be strongly positive for *M. pneumoniae*.

Daily oral azithromycin (500 mg) was added to the antibiotic regimen. The knee symptoms improved substantially two weeks after the azithromycin was started. She completed six weeks of azithromycin therapy and was then monitored while she was off all antibiotics for twelve weeks; there was no recurrence of symptoms. She had implantation of a new total knee prosthesis in July 2010, six months after the joint explantation. *M. pneumoniae* PCR of synovial fluid and deep tissue obtained after the reimplantation was weakly positive. She was again treated with IV vancomycin and ertapenem for six weeks and oral azithromycin for two months postoperatively. All antibiotic therapy was then stopped. IV immunoglobulin infusions were continued by the patient's immunologist. At a six-month follow-up evaluation, after she had been off antibiotics for four months, her condition was stable and she had had no recurrent symptoms.

## Discussion

We report a case of periprosthetic joint infection caused by *M. pneumoniae* diagnosed with 16S rRNA gene amplification and sequencing in a patient with hypogammaglobulinemia. The hypogammaglobulinemia was most likely due to treatment for JRA. There are a few reports of periprosthetic joint infection caused by *Mycoplasma hominis*<sup>1</sup>, but, to the best of our knowledge, this is the first report of periprosthetic joint infection attributable to *M. pneumoniae*.

Mycoplasmas are the smallest self-replicating organisms that are capable of cell-free existence. Mycoplasmas are mucosal pathogens, usually in the respiratory or urogenital tract. Native joint infections from mycoplasma species are rare. Other than *M. pneumoniae*<sup>2-5</sup>, mycoplasma species isolated from areas of septic arthritis of native joints include *M. hominis*<sup>5</sup>, *Mycoplasma genitalium*<sup>2</sup>, *Mycoplasma salivarium*<sup>5,6</sup>, and *Ureaplasma urealyticum*<sup>7</sup>. *M. hominis* has also been found in patients with prosthetic joints<sup>1</sup>.

Arthritis of native joints due to mycoplasma species has been described primarily in adults with hypogammaglobulinemia<sup>3,7</sup> and in children with or without hypogammaglobulin-

emia<sup>3,4</sup>. In these individuals, the immune system responds poorly and cannot eliminate mycoplasma infection. Subsequently, extrapulmonary complications can result, with or without preceding pneumonia.

The authors of one study of eighteen patients with primary antibody deficiency and severe, chronic, culture-negative arthritis found, by culturing synovial fluid and biopsy material for mycoplasma, that mycoplasma infection was the most commonly identified cause<sup>8</sup>. Most patients presented with a monoarthritis, usually involving a large joint such as the knee, shoulder, elbow, or hip. The joint fluid was usually yellow and turbid or purulent. If diagnosis and adequate treatment had been delayed, widespread disease often developed that involved multiple joints. None of the patients were systemically ill at presentation, in contrast to the usual clinical picture in acute bacterial septic arthritis<sup>5</sup>. In our literature search, we found only one published report of *M. pneumoniae* causing native knee and wrist joint infection in an adult with normal levels of immunoglobulins; this joint infection was identified two weeks after an atypical pneumonia<sup>8</sup>.

The diagnosis of mycoplasma infection is challenging. Mycoplasma species lack a cell wall and are not detectable by Gram staining. Routine laboratory methods used in the culture of joint fluid do not support the growth of mycoplasma species. Unfortunately, there are no widely available diagnostic tests that allow reliable, rapid diagnosis of *M. pneumoniae* directly from synovial fluid. Thus, a high clinical suspicion is essential for early treatment of mycoplasma infections; however, the diagnosis is often made in retrospect.

Serology continues to be the mainstay of laboratory diagnosis of *M. pneumoniae* infection, with a fourfold increase in titer on enzyme immunoassay of paired acute and convalescent sera or a single high anti-mycoplasma complement fixation antibody titer of >1:128 indicating the presence of the infection<sup>9</sup>. IgM antibodies typically appear early in most microbial infections and have a short half-life. However, reinfection with *M. pneumoniae* is common, and IgM response in reinfections is either minimal or undetectable<sup>10</sup>. When available, PCR performed on respiratory samples, which can be done rapidly and has a high specificity, may be helpful, especially when combined with serology. The sensitivity of PCR decreases as the duration of symptoms and the time to sample collection increase, with a peak sensitivity of 48% at days 0 to 21<sup>11</sup>. Our patient initially had a negative *M. pneumoniae* PCR from a nasopharyngeal swab, but subsequent PCR of the bronchoalveolar lavage collected days later was positive. The negative PCR could have been due to the variability of the collection technique or could have reflected a relative difference in organism titers on the days these samples had been collected.

16S rRNA gene amplification and sequencing can be useful for bacterial identification and for discovery of novel bacteria. The 16S gene is present in almost all bacteria, and 16S rRNA gene sequencing is particularly useful in expediting the identification of slow-growing bacteria, which may take many weeks to grow in culture or to exhibit relevant biochemical reactions for identification purposes<sup>12</sup>; in identification of a

novel bacterial genus and species<sup>13</sup>; and, most importantly, in detection of bacteria that cannot be grown on culture, such as mycoplasma species<sup>13</sup>. Currently, 16S rRNA gene amplification performed directly on clinical specimens is not widely available in most hospital settings. It is performed on a research basis in some centers and is performed by some reference laboratories, although the acceptable sample types for analysis vary by laboratory.

To the best of our knowledge, ours is the first case report of *M. pneumoniae* periprosthetic joint infection. A diagnosis of mycoplasma infection should be considered in patients with hypogammaglobulinemia and culture-negative septic arthritis who do not respond to traditional antibiotics, especially if patients have recently had upper respiratory symptoms. Patients with delayed diagnosis may sustain considerable joint damage. Treatment for mycoplasmal arthritis is usually azithromycin<sup>3</sup> or tetracycline<sup>5</sup>; prolonged treatment may be required

(three months to two years)<sup>5,14</sup>. Intramuscular immunoglobulin or IV immunoglobulin can be considered for severe infection<sup>5</sup>. ■

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