# Washington University School of Medicine Digital Commons@Becker

**Open Access Publications** 

9-21-2011

# Mycoplasma pneumoniae periprosthetic joint infection identified by 16S ribosomal RNA gene amplification and sequencing: A case report

Zhuolin Han

Washington University School of Medicine in St. Louis

Carey-Ann D. Burnham

Washington University School of Medicine in St. Louis

John Clohisy

Washington University School of Medicine in St. Louis

Hilary Babcock

Washington University School of Medicine in St. Louis

Follow this and additional works at: http://digitalcommons.wustl.edu/open access pubs



Part of the Medicine and Health Sciences Commons

### Recommended Citation

Han, Zhuolin; Burnham, Carey-Ann D.; Clohisy, John; and Babcock, Hilary, ,"Mycoplasma pneumoniae periprosthetic joint infection identified by 16S ribosomal RNA gene amplification and sequencing: A case report." The Journal of Bone and Joint Surgery.93,18. e103.1-e103.3. (2011).

http://digitalcommons.wustl.edu/open\_access\_pubs/1037

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.

Copyright © 2011 by The Journal of Bone and Joint Surgery, Incorporated

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

## A Case Report

Zhuolin Han, MD, MS, Carey-Ann D. Burnham, PhD, John Clohisy, MD, and Hilary Babcock, MD, MPH

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

hronic, 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

**Disclosure:** None of the authors received payments or services, either directly or indirectly (i.e., via his or her institution), from a third party in support of any aspect of this work. One or more of the authors, or his or her institution, has had a financial relationship, in the thirty-six months prior to submission of this work, with an entity in the biomedical arena that could be perceived to influence or have the potential to influence what is written in this work. No author has had any other relationships, or has engaged in any other activities, that could be perceived to influence or have the potential to influence what is written in this work. The complete **Disclosures of Potential Conflicts of Interest** submitted by authors are always provided with the online version of the article.

THE JOURNAL OF BONE & JOINT SURGERY JBJS.ORG VOLUME 93-A · NUMBER 18 · SEPTEMBER 21, 2011 M. PNEUMONIAE PERIPROSTHETIC JOINT INFECTION IDENTIFIED BY GENE AMPLIFICATION AND SEQUENCING

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>5</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 mycoplasmal infection was the most commonly identified cause<sup>5</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 10. 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 2111. 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

THE JOURNAL OF BONE & JOINT SURGERY · JBJS.ORG VOLUME 93-A · NUMBER 18 · SEPTEMBER 21, 2011 M. PNEUMONIAE PERIPROSTHETIC JOINT INFECTION IDENTIFIED BY GENE AMPLIFICATION AND SEQUENCING

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 azithromycin³ or tetracycline⁵; 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>. ■

Zhuolin Han, MD, MS
Carey-Ann D. Burnham, PhD
John Clohisy, MD
Hilary Babcock, MD, MPH
Department of Medicine (Z.H. and H.B.), Pediatrics (C.-A.D.B.),
Pathology & Immunology (C.-A.D.B.), and Orthopedics (J.C.),
Washington University School of Medicine, 660 South Euclid Avenue,
Campus Box 8116, St. Louis, MO 63110.

E-mail address for Z. Han: zhan@dom.wustl.edu.

E-mail address for C.-A.D. Burnham: Burnham\_c@kids.wustl.edu.

E-mail address for J. Clohisy: clohisyj@wudosis.wustl.edu.

E-mail address for H. Babcock: hbabcock@im.wustl.edu

#### References

- **1.** Sneller M, Wellborne F, Barile MF, Plotz P. Prosthetic joint infection with Mycoplasma hominis. J Infect Dis. 1986;153:174-5.
- 2. Tully JG, Rose DL, Baseman JB, Dallo SF, Lazzell AL, Davis CP. Mycoplasma pneumoniae and Mycoplasma genitalium mixture in synovial fluid isolate. J Clin Microbiol. 1995:33:1851-5.
- **3.** Azumagawa K, Kambara Y, Murata T, Tamai H. Four cases of arthritis associated with Mycoplasma pneumoniae infection. Pediatr Int. 2008;50:511-3.
- **4.** Schwartz R, Garty BZ. Variability of arthritis associated with mycoplasma pneumoniae infection in children. Clin Pediatr (Phila). 2005;44:633-6.
- **5.** Franz A, Webster AD, Furr PM, Taylor-Robinson D. Mycoplasmal arthritis in patients with primary immunoglobulin deficiency: clinical features and outcome in 18 patients. Br J Rheumatol. 1997;36:661-8.
- So AK, Furr PM, Taylor-Robinson D, Webster AD. Arthritis caused by Mycoplasma salivarium in hypogammaglobulinaemia. Br Med J (Clin Res Ed). 1983;286:762-3.
- 7. Sordet C, Cantagrel A, Schaeverbeke T, Sibilia J. Bone and joint disease associated with primary immune deficiencies. Joint Bone Spine. 2005;72:503-14.
- **8.** Davis CP, Cochran S, Lisse J, Buck G, DiNuzzo AR, Weber T, Reinarz JA. Isolation of Mycoplasma pneumoniae from synovial fluid samples in a patient with pneumonia and polyarthritis. Arch Intern Med. 1988;148:969-70.

- **9.** Sánchez-Vargas FM, Gómez-Duarte OG. Mycoplasma pneumoniae-an emerging extra-pulmonary pathogen. Clin Microbiol Infect. 2008;14:105-17.
- **10.** Sillis M. The limitations of IgM assays in the serological diagnosis of Mycoplasma pneumoniae infections. J Med Microbiol. 1990;33:253-8.
- 11. Thurman KA, Walter ND, Schwartz SB, Mitchell SL, Dillon MT, Baughman AL, Deutscher M, Fulton JP, Tongren JE, Hicks LA, Winchell JM. Comparison of laboratory diagnostic procedures for detection of Mycoplasma pneumoniae in community outbreaks. Clin Infect Dis. 2009;48:1244-9
- **12.** Heller R, Jaulhac B, Charles P, De Briel D, Vincent V, Bohner C, Piémont Y, Monteil H. Identification of Mycobacterium shimoidei in a tuberculosis-like cavity by 16S ribosomal DNA direct sequencing. Eur J Clin Microbiol Infect Dis. 1996;15: 172-5.
- **13.** Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect. 2008;14:908-34.
- **14.** O'Sullivan MV, Isbel NM, Johnson DW, Rencken K, Byrne S, Graham MM, Playford EG. Disseminated pyogenic Mycoplasma pneumoniae infection in a renal transplant recipient, detected by broad-range polymerase chain reaction. Clin Infect Dis. 2004;39:e98-9.