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Ureaplasma parvum Prosthetic Joint Infection Detected by PCR

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We describe the first reported case of *Ureaplasma parvum* prosthetic joint infection (PJI) detected by PCR. *Ureaplasma* species do not possess a cell wall and are usually associated with colonization and infection of mucosal surfaces (not prosthetic material). *U. parvum* is a relatively new species name for certain serovars of *Ureaplasma urealyticum*, and PCR is useful for species determination. Our patient presented with late infection of his right total knee arthroplasty. Intraoperative fluid and tissue cultures and pre- and postoperative synovial fluid cultures were all negative. To discern the pathogen, we employed PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS). Our patient's failure to respond to empirical antimicrobial treatment and our previous experience with PCR/ESI-MS in culture-negative cases of infection prompted us to use this approach over other diagnostic modalities. PCR/ESI-MS detected *U. parvum* in all samples. *U. parvum*-specific PCR testing was performed on all synovial fluid samples to confirm the *U. parvum* detection.

CASE REPORT

A morbidly obese, 75-year-old non-insulin-dependent diabetic man with a history of nonobstructing renal calculi (incidentally appreciated on serial imaging studies but not further evaluated or treated), hypertension, obstructive sleep apnea, benign prostatic hypertrophy, right hemicolectomy for resection of cecal adenocarcinoma, anal stenosis with chronic laxative dependence, recent-onset atrial fibrillation, and degenerative arthritis of the right knee presented for evaluation of right knee pain and swelling 20 months following right total knee arthroplasty (TKA). He reported discomfort in the right knee of a 1-week duration associated with increased swelling. The patient denied fevers, chills, night sweats, dysuria or hematuria, flank pain, or any other symptoms of systemic infection. He denied a history of gout or trauma to the knee. He did not have any allergies to medications. He did not smoke and was not employed. He led a sedentary lifestyle and was unable to leave his house without a lift device and specialized transport vehicle; he had not been sexually active for several years. On examination, he was afebrile, and his vital signs were within normal limits. He weighed 148.5 kg (body mass index, 47). Examination was remarkable only for a moderate right knee effusion associated with tenderness to palpation; there was a well-healed anterior surgical incision over the joint. Complete blood count revealed 10,200 leukocytes/μl (77% neutrophils). Radiographically, the right TKA appeared well placed and well fixed. There was evidence of a large joint effusion, with no findings of prosthesis loosening or wear and no osseous abnormalities.

Arthrocentesis revealed cloudy synovial fluid with a leukocyte count of 83,792 cells/μl (99% neutrophils) and 50,000 red blood cells (RBCs)/μl. No organisms were appreciated on Gram stain, and aerobic, anaerobic, and fungal cultures of both synovial fluid and blood were all negative. Empirical antimicrobial treatment with vancomycin and cefepime was initiated, and irrigation and debridement of the right TKA were performed the following day. Intraoperatively, copious fluid was noted in the joint. Synovial fluid specimens were submitted for aerobic, anaerobic, fungal, and mycobacterial cultures. Polyethylene implants were removed, and after multiple irrigations with pulsatile lavage, the wound was sprinkled with 2 g of vancomycin powder, and new polyethylene implants were placed. On postoperative day 4, all cultures remained negative, and empirical antimicrobial treatment was changed to vancomycin and ertapenem. The following day, the patient was transferred to a skilled nursing facility to complete a 6-week course of vancomycin and ertapenem treatment.

Two weeks after hospital discharge, the right knee remained warm and swollen and the incision intact. Four weeks postoperatively, the right knee exam was unchanged; and C-reactive protein (CRP) blood tests obtained weekly following surgery did not demonstrate a trend toward normalization that would be expected in response to appropriate antimicrobial treatment (Table 1). A repeat arthrocentesis was performed. Gram stain and aerobic and anaerobic cultures of the synovial fluid were negative. Our prior experience with culture-negative infections prompted us perform PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) on all surgical tissue and synovial fluid samples (1). On postoperative day 48, the CRP remained elevated; based on the results of *Ureaplasma* PCR and PCR/ESI-MS testing described below, the patient's antimicrobial therapy was changed to oral doxycycline. One month later, the infection appeared to have completely resolved. After almost 3 months of doxycycline treatment, our patient reported significant improvement in right TKA pain and swelling. A small effusion was appreciated on exam, and a final arthrocentesis was performed (Table 1). *Ureaplasma* pros-

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TABLE 1 Culture versus *Ureaplasma parvum* PCR and PCR/ESI-MS results

Day	Sample	Gram stain result	Culture result	PCR/ESI-MS result	PCR level (genome equivalents/ well)	*U. parvum* PCR result	CRP level (mg/dl)	Antimicrobial treatment (no. of days of treatment)
−1	Synovial fluid	No organisms seen	No growth	*U. parvum*	340	Positive	8.27	None
Day of surgery	Synovial fluid	No organisms seen	No growth	*U. parvum*	366		8.83	Vancomycin (1)
	Articular tissue	No organisms seen	No growth	*U. parvum*	234			Cefepime (1)
POD[a]								
28	Synovial fluid	No organisms seen	No growth	*U. parvum*	35	Positive	3.15	Vancomycin (29), ertapenem (24)
48[b]							3.33	Vancomycin (49), ertapenem (44)
132	Synovial fluid	No organisms seen	No growth	No detection	NA[c]	Negative	1.97	Doxycycline (84)

[a] POD, postoperative day.
[b] On day 48, antibiotic treatment was changed to oral doxycycline based on the results of the PCR and PCR/ESI-MS testing.
[c] NA, not available.

thetic joint infections (PJIs) are associated with significant morbidity, and relapse of infection has been described (1, 2). Due to our patient's multiple comorbidities, an extended course of doxycycline treatment is planned.

TKA infections can be devastating, with significant mortality and cost. The Centers for Disease Control and Prevention's National Nosocomial Infections Surveillance (NNIS) system has reported that between 0.5 and 1.4% of TKA surgeries are complicated by complex or deep joint infection, and up to 3% of patients experience some type of postoperative infectious complication (3). In a prospective study of TKA infections with 12 months of follow-up, Levent et al. identified obesity as a significant risk factor for infection following TKA, and pathogen detection by culture of synovial fluid was diminished by preoperative empirical antibiotic treatment (4).

Despite our best efforts to increase diagnostic yield, such as implant sonication, there remain cases of culture-negative PJI (5). In the selected cases of culture-negative PJI not responding to empirical antimicrobial treatment, detection of pathogens by using specialized microbiologic assays may be useful (6–8). PCR/ESI-MS has demonstrated the capacity to detect pathogens in clinical samples obtained following initiation of antimicrobial treatment (9).

PCR/ESI-MS does not depend on specific amplification of any one bacterial target. Multiple broad primer pairs used in the assay amplify >800 clinically relevant and diverse bacteria, one or more of which might be present in any given sample (10). Nine primer pairs used in eight wells (including one duplex) are targeted to broadly conserved bacterial loci, including four primer pairs that amplify segments of the 16S and 23S ribosomal genes (targeting all bacterial species) and five pairs targeting housekeeping genes (one pair each for *rplB*, *tufB*, and *valS* and two for *rpoB*) of specific subgroups of bacteria to provide species-level resolution (11). The broad ribosomal primer pairs used in the assay amplify all *Ureaplasma* species. The amplification products are analyzed in an unbiased manner using a mass spectrometer.

Despite antimicrobial treatment following surgical débridement with polyresin exchange, our patient's knee pain did not improve, and the CRP level remained elevated, prompting further testing of right knee fluid. Although our patient had a history of kidney stones, and *Ureaplasma* strains have a well-known association with urinary tract infection (UTI) in the setting of nephrolithiasis, our patient had no history of systemic bacterial infection (in general) or *Ureaplasma* infection (in particular). Furthermore, there is only one reported case of *Ureaplasma* PJI, a *Ureaplasma urealyticum* prosthetic hip infection (although *Ureaplasma parvum* has been reported in the setting of native joint polymicrobic infection in a profoundly immunocompromised patient) (1, 2).

Synovial fluid was submitted for testing by PCR/ESI-MS, as described by Kaleta et al. (10) PCR/ESI-MS detected *U. parvum* from synovial fluid obtained by arthrocentesis as well as fluid and tissue obtained in the operating room (OR) after empirical antibiotics had been administered (Table 1). *U. parvum* detection was confirmed by a *U. parvum*-specific qualitative PCR assay (7). PCR/ESI-MS again detected *U. parvum* in synovial fluid obtained on postoperative day 28, prior to initiation of antimicrobial treatment with doxycycline (Table 1), which was also confirmed by *U. parvum*-specific PCR (7). A decline in the level of detection of *U. parvum* by PCR/ESI-MS was noted in the synovial fluid specimen obtained on postoperative day 28, and following 2 months of oral doxycycline treatment, neither PCR/ESI-MS nor *U. parvum*-specific PCR detected *U. parvum* in synovial fluid from the right knee (Table 1). Finally, using the 16S rRNA primer pairs FDA-16S-4F (5′-TTGGAGAGTTTGATCCTGGCTC-3′) and 16S-801R-2 (5′-GGCGTGGACTACCAGGGTATCT-3′) in a conventional PCR of DNA extracted from synovial fluid and articular tissue collected in the OR, a 760-bp PCR product was amplified and sequenced on an ABI machine: AGGATTAACGCTGGCGGCATGCCTAATACATGCAAATCGAACGAAGCCTTTTAGGCTTAGTGGTGAACGGGTGAGTAACACGTATCCAATCTACCCTTAAGTTGGGGATAACTAGTCGAAAGATTAGCTAATACCGAATAATAACATCAATATCGCATGAGAAGATGTAGAAAGTCGCTCTTTGTGGCGACGCTTTTGGATGAGGGTGCGACGTATCAGATAGTTGGTGAGGTAATGGCTCACCAAGTCAATGACGCGTAGCTGTACTGAGAGGTAGAACAGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGGGAATTTTTCACAATGGGCGCAAGCCTTATGAAGCAATGCCGCGTGAACGATGAAGGTCTTATAGATTGTAAAGTTCTTTTATATGGGA

TABLE 2 NCBI-BLAST results for preoperative synovial fluid specimens

GenBank sequence description	GenBank sequence accession no.	GenBank sequence length (bp)	Identity, bp (%)	No. (%) of gaps/total bp
U. parvum serovar 3 ATCC 700970 16S rRNA, complete genome	NR_074176.1	1,466	760/760 (100)	0/760 (0)
U. parvum serovar 3 ATCC 27815 16S rRNA, complete genome	NR_074762.1	1,531	760/760 (100)	0/760 (0)
U. parvum serovar 3 ATCC 27815 16S rRNA, partial sequence	NR_027532.1	1,439	760/760 (100)	0/760 (0)

AGAAACGCTAAGATAGGAAATGATTTTAGTTTGACTGTAC CATTTGAATAAGTATCGGCTAACTATGTGCCAGCAGCCG CGGTAATACATAGGATGCAAGCGTTATCCGGATTTACTG GGCGTAAAACGAGCGCAGGCGGGTTTGTAAGTTTGGTAT TAAATCTAGATGCTTAACGTCTAGCTGTATCAAAAACTGT AAACCTAGAGTGTAGTAGGGAGTTGGGGAACTCCATGTG GAGCGGTAAAATGCGTAGATATATGGAAGAACACCGGTG GCGAAGGCGCCAACTTGGACTATCACTGACGCTTAGGCT CGAAAGTGTGGGGAGCAAATAGGAT. A homology search carried out by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) confirmed the *U. parvum* 16S rRNA gene sequence (Table 2).

The implications of this case are clear: molecular methods capable of identifying unusual or unanticipated pathogens directly from specimens associated with prosthetic joints may provide an opportunity to direct antibiotic treatment against pathogens not responding to empirical antimicrobial treatment. PCR/ESI-MS may have a role as an adjunct to conventional diagnostic microbiologic methods in culture-negative PJIs, even for *Ureaplasma* spp., which can be readily cultured in appropriate media (e.g., A8 agar and 10B arginine broth), allowing for antimicrobial susceptibility testing.

The 2013 clinical practice guidelines for diagnosis and management of PJIs do not include recommendations or suggestions for the role of molecular diagnostics in the approach to culture-negative PJIs (12). As diagnostic modalities such as PCR/ESI-MS and *Ureaplasma*-specific PCR are used more commonly, the detection of *Ureaplasma* spp. in PJIs may increase, thereby changing our approach to culture-negative infections and our understanding of syndromes caused by pathogens that are devoid of cell walls. The prevalence of PJIs caused by *Ureaplasma* spp. and the role of pathogen-specific PCR and/or PCR/ESI-MS in diagnosis and monitoring response to treatment versus more affordable methods, such as culture, that provide an opportunity for antimicrobial susceptibility testing need to be defined. Future prospective investigations, appropriately powered to analyze the diagnostic accuracy and utility of species-specific PCR and PCR/ESI-MS for PJI diagnosis, should be performed and guidelines updated to reflect these data.

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