

CASE REPORTS

Ureaplasma urealyticum Continuous Ambulatory Peritoneal Dialysis-Associated Peritonitis Diagnosed by 16S rRNA Gene PCR[∇]

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Received 25 May 2010/Returned for modification 9 July 2010/Accepted 17 August 2010

In some patients with peritonitis complicating continuous ambulatory peritoneal dialysis (CAPD), a causative organism is never identified. We report a case of *Ureaplasma urealyticum* CAPD-associated peritonitis diagnosed by 16S rRNA gene PCR. *Ureaplasma* may be an underrecognized cause of peritonitis because it cannot be recovered using routine culture methods.

CASE REPORT

A 28-year-old woman with a history of lupus nephritis and end-stage renal disease on peritoneal dialysis was evaluated for recurrent peritonitis. The patient was born in India, recalled receiving *Mycobacterium bovis* BCG vaccination, and moved to the United States at age 14. A purified protein derivative (PPD) test was negative in 2007. The patient was diagnosed with systemic lupus erythematosus at age 18 and began peritoneal dialysis at age 26. Her past medical history was also notable for an appendectomy in the remote past, hypertension, and a history of congenital bullous lung disease for which she underwent bullectomy 2 years prior to admission. Six months prior to admission, she developed a lung abscess at the site of her prior resection. She said she did not smoke, drink alcohol, or use recreational drugs and was not sexually active.

The patient had been hospitalized twice during the preceding year for culture-negative peritonitis, initially 12 months and then 6 months prior to her current admission. During the admission 6 months prior to admission, the patient had presented with fever, abdominal pain, and cloudy dialysate. An initial sample from the patient's peritoneal fluid revealed 77 nucleated cells with 57% neutrophils; a repeat sample taken a few hours later showed 145 nucleated cells with 56% nucleated cells. Given the patient's clinical presentation and dialysate findings, the patient was diagnosed with peritonitis and was initiated on intravenous (i.v.) vancomycin and piperacillin-tazobactam and intraperitoneal vancomycin and ceftazidime. Over the course of that admission, the patient was also found

to have a lung abscess at the site of her prior bullectomy, and *Clostridium difficile* colitis for which she received oral metronidazole. The lung abscess was not drained, and no specific microbiologic etiology for the abscess was identified; the patient was felt to be receiving appropriate treatment with the broad-spectrum antibiotics she was receiving for her peritonitis. A tunneled line was inserted, and the patient's treatment was changed to ertapenem on her fourth day of therapy for ease of home dosing. She received a total of 10 weeks of i.v. antibiotics, accompanied by ongoing oral metronidazole for prophylaxis of recurrent *Clostridium difficile* infection, until she demonstrated radiographic resolution of her abscess. Two weeks prior to admission, she had another episode of peritonitis, characterized by abdominal pain, nausea, vomiting, low-grade fever, and cloudy dialysate. Because she had previously had a superficial swab of her catheter exit site that had been positive for methicillin-resistant *Staphylococcus aureus* (MRSA), the patient was treated with intraperitoneal vancomycin only. She experienced transient improvement but required readmission twice over the subsequent 10-day period for the same symptoms. She said she did not have diarrhea. Her medications included hydroxychloroquine, mycophenolic acid, prednisone (5 mg daily), and losartan.

On examination, the patient was afebrile but in some distress from her abdominal pain. She had a peritoneal catheter in place with no local evidence of inflammation; her abdominal exam was further notable for hypoactive bowel sounds, guarding, diffuse pain to light palpation throughout the abdomen, and rebound tenderness. Laboratory testing revealed a peripheral blood leukocytosis of 12,400/mm³ with 95% neutrophils. Sampling of the dialysate revealed 1,611 nucleated cells (76% neutrophils, 22% monocytes, and 1% eosinophils) and 39 red blood cells per mm³. Samples of peritoneal fluid were cultured for bacteria, fungi, and acid-fast bacilli. Over the next several days, the patient failed to show significant clinical improve-

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[∇] Published ahead of print on 25 August 2010.

ment. By her fourth day in the hospital, her leukocytosis had increased to 21.9×10^3 cells/mm³, with an erythrocyte sedimentation rate (ESR) of 89 mm/h and a C-reactive protein level of 194 mg/liter. Blood and peritoneal fluid cultures from the current and previous admissions remained negative, as did urine nucleic acid amplification tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. A serum anti-double-stranded DNA (anti-dsDNA) level was found to be strongly positive at 207 IU/ml. On hospital day four, the patient underwent removal of her peritoneal dialysis catheter and was transitioned to hemodialysis. Following removal of her catheter, *Ureaplasma urealyticum* 16S rRNA gene sequences were detected by PCR in two separate samples of the patient's peritoneal fluid. Total DNA from peritoneal fluid samples was extracted with a High Pure PCR Template preparation kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions, and the 16S rRNA gene was sequenced as previously described (6). Briefly, template DNA was PCR amplified in a reaction mixture containing 1× AmpliTaq buffer, 3 mM MgCl₂, 200 μM each deoxynucleoside triphosphate (dNTP), primers at 1 μM each, and 1.25 units of AmpliTaq enzyme (Applied Biosystems, Foster City, CA). An initial incubation at 95°C for 10 min was followed by denaturation at 95°C for 30 s, annealing at 68°C for 30 s, and extension at 72°C for 1 min 15 s. After 30 cycles (1 cycle consisting of denaturation, annealing, and extension) were completed, a 10-min extension at 72°C ensured completely double-stranded amplification products. The resulting amplicons were purified using a Ultracel YM-100 ultrafiltration unit (Millipore, Billerica, MA) according to the manufacturer's recommendations. Both strands were sequenced using the BigDye sequencing kit (Applied Biosystems, Foster City, CA) and assembled into a double-stranded contig using Sequencher software (Genecode, Ann Arbor, MI). The final sequence was used to search the NCBI sequence database using BLAST. Identification of *Ureaplasma urealyticum* in the peritoneal fluid was confirmed: the sequence obtained was 100% identical over 260 bp to several *Ureaplasma urealyticum* sequences (e.g., *U. urealyticum* ATCC 33699, GenBank accession no. CP001184.1) but only 96% identical to a closely related species, *Ureaplasma parvum* (e.g., *U. parvum* ATCC 700970, GenBank accession no. AF222894.1) (7). Following PCR identification, no further attempt was made to culture the organism on specific media. Treatment was initiated with doxycycline (100 mg per os [p.o.] twice a day [BID]) for 14 days. The patient had slow but complete resolution of her symptoms without clinical evidence of relapse at 6 months.

Comment. Peritonitis remains a serious cause of morbidity and mortality in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Surveys indicate that peritonitis is the major clinical complication of peritoneal dialysis and the most common reason for discontinuation of CAPD (2). Rapid diagnosis and treatment of these infections are critical for both preservation of the catheter and resolution of the symptoms. Although conventional microbiology is able to successfully recover organisms in greater than 80% of cases (12), many cases are still categorized as "culture negative" (3), and causative organisms cannot be recovered in nearly a third of CAPD

patients with relapsing peritonitis (11). Recommendations for empirical therapy include broad coverage for both Gram-positive and Gram-negative organisms (8), as infection commonly results from either contaminating skin flora or enteric organisms.

Fastidious organisms, such as *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, or genital mycoplasmas, have been isolated occasionally from patients with CAPD-related peritonitis (1). Detection of these organisms can have important implications for treatment. For example, tetracyclines and macrolides are generally regarded as the agents of choice for infections caused by *Ureaplasma urealyticum* but are seldom included in empirical regimens for peritonitis. Failure to detect fastidious agents can place patients at risk for complications of infection and broad-spectrum antibiotics, the loss of CAPD access, and superinfection with *Clostridium difficile*, as occurred in our patient.

Molecular methods can improve diagnostic sensitivity in CAPD-associated peritonitis (13) and facilitate the detection of fastidious organisms, including *U. urealyticum* (1). This report represents only the third case of *Ureaplasma* peritonitis in the literature (1, 5), and the second seen in association with CAPD. Interestingly, the previously reported CAPD-associated case also occurred in a young woman with lupus nephritis, following insertion of an intrauterine device. *Ureaplasma urealyticum* and the closely related species *U. parvum* colonize the lower genital tract mucosa of 40 to 80% of sexually active women and less than 10% of adults who are not sexually active, such as our patient. The organism may have gained access to the peritoneum via the Fallopian tubes in our patient. Although ureaplasmas can be cultivated using mycoplasma media, such as A8 agar, detection is increasingly made by molecular techniques (10). *U. urealyticum* and *U. parvum* can be distinguished only by molecular methods.

Diagnosis in our patient was particularly challenging because of the possibilities of chemical peritonitis from the administration of intraperitoneal antibiotics or lupus-related serositis. The presence of leukocytosis provided an important clue that lupus was not the cause of the ongoing peritoneal inflammation, as lupus flares are typically associated with low peripheral white blood cell counts. Genital mycoplasmas, including *U. urealyticum* have been reported to be more prevalent in urine specimens from patients with lupus (4), although subsequent studies have reached conflicting conclusions in this regard (9). The present report demonstrates the ability of *U. urealyticum* to cause relapsing peritonitis in the setting of CAPD and the value of molecular diagnostic methods in this clinical setting.

We did not have any conflicts of interest or receive any financial support related to this work.

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