[CASE REPORT]

***Mycobacterium wolinskyi* Peritonitis after Peritoneal Catheter Embedment Surgery**

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Abstract:

Mycobacterium wolinskyi belongs to the *Mycobacterium smegmatis* group, which comprises rapidly growing non-tuberculous mycobacteria. The number of case reports on *M. wolinskyi* infections associated with postoperative wounds has increased in recent years. We herein report a case of peritonitis due to *M. wolinskyi* after peritoneal catheter embedment surgery. Identification was achieved based on 16S ribosomal RNA and *rpoB* gene sequencing of the isolate. The patient recovered following catheter removal and treatment with levofloxacin and minocycline for one month.

Key words: 16S rRNA sequence, *Mycobacterium wolinskyi*, non-tuberculous mycobacteria, peritoneal dialysis, rapidly growing mycobacteria, *rpoB* sequence

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Introduction

Peritoneal dialysis (PD) is an important modality for the management of end-stage renal disease (ESRD). Infection is a serious complication that may result in cessation of PD and catheter loss (1-3). Various efforts have been undertaken to decrease the incidence of infectious complications of PD and peritoneal catheter embedment; the Moncrief and Popovich technique represents one successful procedure (4).

The most common PD-related infectious complication is catheter-associated peritonitis, of which the causative organisms are usually aerobic skin inhabitants such as *Staphylococcus* species (5). Recently, reports of PD-related peritonitis caused by non-tuberculous mycobacteria (NTM) have increased (6-9). We herein report a case of peritonitis due to *Mycobacterium wolinskyi*, a rare, rapidly growing NTM, after peritoneal catheter embedment surgery via the Moncrief and Popovich technique.

Case Report

A 66-year-old Japanese man with ESRD secondary to diabetic nephropathy was admitted to our hospital with a fever, generalized fatigue, and weight loss of 6 kg over 2 months. Two months prior to admission, he underwent PD catheter insertion by stepwise initiation using the Moncrief and Popovich technique (4). One month prior to admission, he noticed discharge from the surgical site and visited a nearby clinic, where he received wound care with gentamicin ointment. No microbiological evaluation was performed during the visit, and the discharge persisted. Upon admission to our hospital, he complained of tingling pain around the abdominal surgical site with a small amount of discharge. His vital signs were as follows: blood pressure, 136/80 mmHg; pulse, 84 beats per minute; temperature, 36.8°C; and respiratory rate, 12 breaths per minute. On a physical examination, a small amount of serous discharge was noted at the surgical site. His abdomen was not distended. No re-

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Figure. A: Gram staining of colonies revealing Gram-positive bacilli of moderate length (×1,000). B: Ziehl-Neelsen staining of colonies revealing red bacilli (×1,000).

bound tenderness or muscle guarding was observed.

Laboratory findings on admission were as follows: total white blood cell (WBC) count of 12,000 cells/mm³ with 85% neutrophils, 6% lymphocytes, 1% basophils, and 8% monocytes; serum potassium, 6.6 mEq/L; blood urea nitrogen, 167 mg/dL; creatinine, 12.54 mg/dL; C-reactive protein, 17.8 mg/dL; and procalcitonin, 3.29 ng/mL. Both abdominal computed tomography and abdominal echography demonstrated a small amount of ascites without other notable abnormal findings. An intravascular catheter was inserted via the right femoral vein, and emergency hemodialysis was performed. The PD catheter was exteriorized, and cloudy ascites obtained through the catheter showed a WBC count of 2,000 per mm³ nucleated cells (37% neutrophils, 48% lymphocytes, and 14% monocytes).

The discharge and two paired blood cultures were obtained for a microbiological evaluation on admission. Ascitic fluid was inoculated into blood culture bottles (BacT/Alert SA and SN bottles; Sysmex bioMérieux, Tokyo, Japan). No bacteria were observed on the Gram stain of the discharge. Treatment with intravenous ceftriaxone (2 g/day) was commenced and later switched to cefazolin (1 g/day) and ceftazidime (1 g/day), owing to a persistent fever. On the fifth day of hospitalization, small white colonies grew on a sheep blood agar plate (Kyokuto, Tokyo, Japan) from the culture of the discharge; Gram staining demonstrated Gram-positive bacilli of moderate length (Figure A) that were also positive for Ziehl-Neelsen staining (Figure B). The blood cultures were negative. The ascitic fluid in an aerobic blood culture bottle also became positive and grew similar colonies on a sheep blood agar plate. Initially, these organisms were determined as “unidentified Gram-positive rods.” Thirteen days after admission, the PD catheter was removed, and the pus obtained this time grew similar colonies to the ascitic fluid. It was only at 21 days following admission that the laboratory identified these organisms as rapidly growing mycobacteria, at which time the patient’s general condition had improved.

On the 27th day of hospitalization, the susceptibility of the organism, performed via the microdilution method and

interpreted based on Clinical and Laboratory Standards Institute M24 A-2, revealed unexpectedly high minimum inhibitory concentrations toward clarithromycin, imipenem, and tobramycin (Table 1) (10). Identification of the organism by DNA-DNA hybridization (DDH mycobacterium “Kyokuto”; Kyokuto Pharmaceutical Industrial, Tokyo, Japan) indicated the isolation of either *Mycobacterium fortuitum* or *Mycobacterium peregrinum*. As the patient’s condition at that time was favorable, we withheld the commencement of antibiotics until the results of the 16S ribosomal RNA (rRNA) and *rpoB* gene analyses were obtained. We used the EzTaxon-e Database (<http://www.ezbiocloud.net>) and BLAST database (<http://blast.ddbj.nig.ac.jp/blastn>) for the 16S rRNA and *rpoB* gene sequences, respectively. The bacterial 16S rRNA gene sequence detected in the ascitic fluid was 100% (996 bp) identical to *M. wolinskyi* ATCC 700010^T. *M. peregrinum* was the most closely-related species in the 16S rRNA gene sequence (99.6%). The result of the amplified *rpoB* gene sequences (650 bp) showed high similarity (99%) with *M. wolinskyi* ATCC 700010^T, *Mycobacterium jacuzzi*, and *M. wolinskyi* CRM-0267. Thus, the isolate was ultimately identified as *M. wolinskyi*. No other microorganisms grew in the blood cultures or ascites cultures.

Based on the identification and susceptibility results, oral levofloxacin (750 mg, then 500 mg every other day) and minocycline (100 mg twice daily) were administered. Amikacin was not initiated, as the patient’s condition was stable and the surgical site had improved upon removal of the PD catheter. Thirty-nine days later, antibiotics were discontinued due to development of thrombocytopenia. Thereafter, the platelet counts improved. There was no recurrence of a fever or abdominal symptoms at the six-month follow-up.

Discussion

Peritonitis is a major cause of morbidity and mortality in patients receiving PD that may lead to cessation of PD (1-3). The usual causative pathogens of PD-related peri-

Table 1. Antimicrobial Susceptibility Testing for the Isolated *Mycobacterium wolinskyi*.

Antibiotics	MIC (μg/mL)	MIC (μg/mL) for category		
		Susceptible	Intermediate	Resistant
Amikacin	4	≤16	32	≥64
Tobramycin	≥16	≤2	4	≥8
Cefmetazole ^a	8	≤16	32-64	≥128
Imipenem	8	≤4	8-16	≥32
Clarithromycin	≥32	≤2	4	≥8
Minocycline ^b	≤0.25	≤1	2-4	≥8
Ciprofloxacin	0.5	≤1	2	≥4
Moxifloxacin	≤0.5	≤1	2	≥4
Trimethoprim-sulfamethoxazole	≤2/38	≤2/38		≥4/76
Linezolid	3	≤8	16	≥32

The data of breakpoints are derived from ref (10).

a. The breakpoints are derived from those for cefoxitin.

b. The breakpoints are derived from those for doxycycline.

MIC: minimum inhibitory concentration

tonitis are aerobic bacteria such as coagulase negative staphylococci, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*; however, up to 30% of cases are so-called “culture-negative” (2, 5, 6, 11). It is speculated that most culture-negative cases are a result of empirical antibiotic administration prior to the procurement of microbiological specimens; however, pathogens that are difficult to identify, such as fungi and mycobacteria, also cause PD-related peritonitis.

M. wolinskyi belongs to the Runyon classification type IV NTM, also known as rapidly growing mycobacteria (RGM); this means that it has the ability to grow within seven days on artificial media (12). NTM, including RGM, are important emerging pathogens in PD-associated peritonitis. In 2012, Song et al. performed a literature review of 41 articles on PD-associated NTM peritonitis; more than half of the reported cases were due to RGM (*M. fortuitum*: 38.6%; *M. chelonae*: 14%) (7).

RGM are ubiquitous in soil, dust, and water, as well as much of the natural environment. Most reported cases of PD-associated RGM peritonitis developed while patients were on PD, suggesting that the patients may have acquired RGM during PD manipulation. Our patient developed infection soon after catheter embedment surgery via the Moncrief and Popovich technique, and the catheter was not used for dialysis; therefore, our case may well be a surgical site infection. Recently, outbreaks of RGM infection after cardiac surgery have been reported, and the contamination of the water in the heater-cooler unit has been reported (13). Such units are not usually used in urological surgery, and the exact route of infection in our case remains unclear. To date, we have detected only one case of *M. wolinskyi* infection in our hospital.

Due to its rarity and variability in antimicrobial susceptibility, recommendations for specific antibiotic treatment or duration of therapy are lacking. The combination of at least

two antibiotics guided by susceptibility testing is generally recommended (14). The typical *M. wolinskyi* susceptibility profile has been reported as follows: susceptibility towards amikacin, cefoxitin, imipenem, doxycycline, and ciprofloxacin, with resistance towards sulfamethoxazole, clarithromycin, and tobramycin (8, 15). Gentamicin ointment is reportedly effective in the prophylaxis of catheter exit site infections (16). However, cross resistance to gentamicin in tobramycin-resistant organisms is common, and the gentamicin ointment/cream used in our case may have selected *M. wolinskyi* (17). Although a macrolide is often described as a key treatment component for NTM infection, *M. wolinskyi* possesses the *erm* gene, inducing inherent macrolide resistance (18). The length of treatment is determined mainly by the clinical response (3). In a review of NTM peritonitis, the treatment duration ranged between 2 days and 24 months and lasted >1 month in 93.3% of cases (7). We performed a literature review of case reports of *M. wolinskyi* infection (Table 2); however, the study by Brown et al. was excluded because their data for susceptibility testing and treatment were lacking (8, 12, 15, 19-26). Most cases are uniformly resistant towards clarithromycin and tobramycin, and a combination of amikacin, minocycline, doxycycline, ciprofloxacin, levofloxacin, moxifloxacin, and linezolid was used. The total treatment duration was typically 6 months.

We used a combination of levofloxacin and minocycline in this patient, guided by antibiotic susceptibility testing. When we initiated the therapy, the patient was in a stable condition; therefore, amikacin was not included in the regime. Although we planned to continue the antibiotics for six months, the patient only managed to receive treatment for one month due to the development of thrombocytopenia. Fortunately, the patient exhibited no signs of relapse at the six-month follow up. Surgical debridement or removal of foreign material is often necessary in cases of PD-associated peritonitis. Catheter removal was performed in the majority

Table 2. Literature Review of Infection Type, Susceptibility, and Treatment of *Mycobacterium wolinskyi* infection.

Reference	Infection type	Susceptibility of <i>M. wolinskyi</i>	Antibiotics and treatment duration	Surgical procedure
(19)	Hip prosthetic infection	S: AMK, CPFX, IPM, LZD, MFLX, MINO, OFLX I: CXT R: CAM, TOB	AMK+MFLX+MINO for 1 month, then MFLX+MINO for 5 months	Debridement
(20)	Bacteremia in a patient with chronic myelogenous leukemia	S: AMK, CPFX, CXT, DOXY, IPM, LVFX, MINO, ST R: CAM, TOB	AMK+MINO+LVFX for 1 month, then MINO+LVFX for 5 months	None
(15)	Bacteremia and multiple joint infection in a patient with non-Hodgkin lymphoma	S: AMK, CPFX, CXT, DOXY, IPM R: CAM, ST, TOB	AMK+MFLX+MINO for 1 month, then MFLX+MINO for 6 months	Debridement
(21)	Surgical site infection after knee replacement arthroplasty for primary osteoarthritis	R: CAM, CXT, IPM, RFP, ST, TOB	AMK+CPFX+DOXY, duration N/A	Debridement
(23)	Facial skin abscess after cosmetic procedures	S: AMK, CPFX, MFLX I: CXT, IPM I or R: CAM R: ST	DOXY+CPFX for 5 months	Drainage and debridement
(22)	Multiple breast abscesses after mammoplasty	S: AMK, CPFX S or I: MFLX (≤ 2) I: CXT, DOXY R: CAM, ST, TOB	AMK+CPFX+DOXY for 10 weeks, then CPFX+DOXY 14 weeks	Drainage and debridement
(8)	Peritonitis in a chronic peritoneal dialysis patient	S: AMK, CPFX, DOXY, LZD, ST	DOXY+LZD+MFLX for 4 weeks	Peritoneal dialysis catheter removal
(24)	Case 1: Sternal wound infection after aortic valve replacement Case 2: Incisional infection after bilateral lung transplant Case 3: Prosthetic graft infection after aortic root replacement Case 4: Pacemaker pocket infection after CABG, aortic valve replacement, and pacemaker placement Case 5: Sternal osteomyelitis after CABG Case 6: Sternal wound infection after aortic valve replacement	NA	Case 1: IPM+MFLX+ST for 1 month, then MFLX+ST for 5 months Case 2: DOXY+MFLX for 3 months Case 3: AMK+DOXY+MFLX for 1 month, then DOXY+MFLX+ST for 6 months, then ST lifelong Case 4: CPFX+MINO for 6 months Case 5: DOXY+MINO for 6 months Case 6: NA, debridement	Case 1: debridement Case 2: debridement Case 3: graft replacement Case 4: pacemaker removal Case 5: debridement with sternectomy Case 6: debridement
(26)	Prosthetic infection after aortic valve replacement for aneurysm of the ascending aorta	NA	AMK+DOXY+LZD+MFLX for 6 months	Replacement of a bio-prosthetic aortic valve and an aortic prosthesis with a vascular homograft.
(25)	Recurrent subcutaneous abdominal wall abscesses and ulcer after insulin injection	S: CPFX, LVFX, LZD, MINO, FLX I: IPM R: CAM, DOXY, TOB	AMK+MFLX+MINO for 1 month, then MFLX+MINO for 5 months	Debridement

AMK: amikacin, CABG: coronary artery bypass grafting, CAM: clarithromycin, CPFX: ciprofloxacin, CXT: cefoxitin, I: intermediate, IPM: imipenem/cilastatin, LVFX: levofloxacin, LZD: linezolid, MFLX: moxifloxacin, MINO: minocycline, NA: not available, OFLX: ofloxacin, R: resistant, S: susceptible, ST: sulfamethoxazole, TOB: tobramycin

of reported cases of NTM peritonitis, as in our case (7).

Conventional identification methods such as DDH may not identify rare *Mycobacterium* species. Therefore, stepwise identification using 16S rRNA sequence and *rpoB* gene analyses is recommended (27). Our patient is the second reported case of PD-related peritonitis due to *M. wolinskyi*; however, there may have been other potential cases in previous reports of *M. fortuitum* or other rare *Mycobacterium* species that were identified by conventional methods (8, 9). As

mentioned, *M. wolinskyi* is typically resistant to clarithromycin and tobramycin, both of which can be used for the treatment of RGM infections. Furthermore, as RGMs can grow in usual blood culture plates and are stained by Gram staining, clinical microbiology laboratories sometimes confuse RGMs for unidentified Gram-positive rods, as in our case. It is therefore crucial to communicate with doctors and clinical microbiologists to facilitate the precise and timely identification with susceptibility testing. The correct identification of

microorganisms is critical, since various NTM species display different antibiotic susceptibility patterns; *M. wolinskyi* is one example that exhibits resistance towards clarithromycin, an antibiotic generally used for the treatment of NTM infections.

In conclusion, we herein reported a case of *M. wolinskyi*-induced peritonitis after peritoneal catheter embedment surgery. Correct identification and appropriate antibiotic susceptibility testing are critical in patient management. Given the increasing trend in numbers of RGM infections, the elucidation of risk factors and preventive measures is warranted.

The authors state that they have no Conflict of Interest (COI).

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