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【 CASE REPORT 】

Mixed Infection of Cytomegalovirus and Pulmonary Nocardiosis Caused by *Nocardia elegans* Diagnosed Using Nanopore Sequencing Technology

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Abstract:
A 69-year-old woman who had undergone renal transplantation and was receiving sulfamethoxazole/trimethoprim (ST) developed pulmonary nocardiosis. To our knowledge, this is the first report of the identification of *Nocardia elegans* using nanopore sequencing, supported by 16S rDNA capillary sequencing findings. Chest computed tomography performed after ST initiation revealed significant improvement of the pulmonary shadows compared to previous findings. We herein report the value of nanopore sequencing for rapid identification of rare pathogens, such as *Nocardia elegans*. Furthermore, our findings suggest that *Nocardia* may infect even patients receiving ST, which is currently the most effective prophylactic drug.

Key words: nocardiosis, nanopore sequencing, immunocompromised, organ transplantation

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Introduction

Nocardia is a Gram-positive, beaded, weakly acid-fast, rod-shaped bacterium found ubiquitously in the soil, which can cause diseases of the lung, central nervous system, and skin in immunocompromised patients (1). More than 80 species have been identified, of which at least 33 are pathogenic in humans. The distribution of various species differs widely depending on the geographic location and generation (2).

Nanopore sequencing is a next-generation sequencing technology in which customized protein nanopores are used to determine nucleic acids and protein sequences (3). Recently, it has been shown to be useful in the detection of several bacterial species (4-7).

We herein report a case of pulmonary nocardiosis caused by *Nocardia elegans* in an immunocompromised patient who had undergone renal transplantation and was taking sulfamethoxazole/trimethoprim (ST) as prophylaxis for *Pneumocystis jirovecii* pneumonia (PCP). To our knowledge, this is the first report of the identification of *Nocardia* in clinical isolates using a nanopore sequencer, supported by findings of 16S rDNA capillary sequencing performed using the classical method.

Case Report

A 69-year-old woman with IgA nephropathy underwent renal transplantation with a kidney donated by her husband. She developed an intermittent nocturnal fever approximately two months after transplantation. However, her diurnal body temperatures were normal. Two weeks after the fever onset, she visited our hospital for postoperative follow-up. She was receiving tacrolimus (3 mg/day), mycophenolate mofetil (1,500 mg/day), prednisolone (10 mg/day), and PCP prophy-

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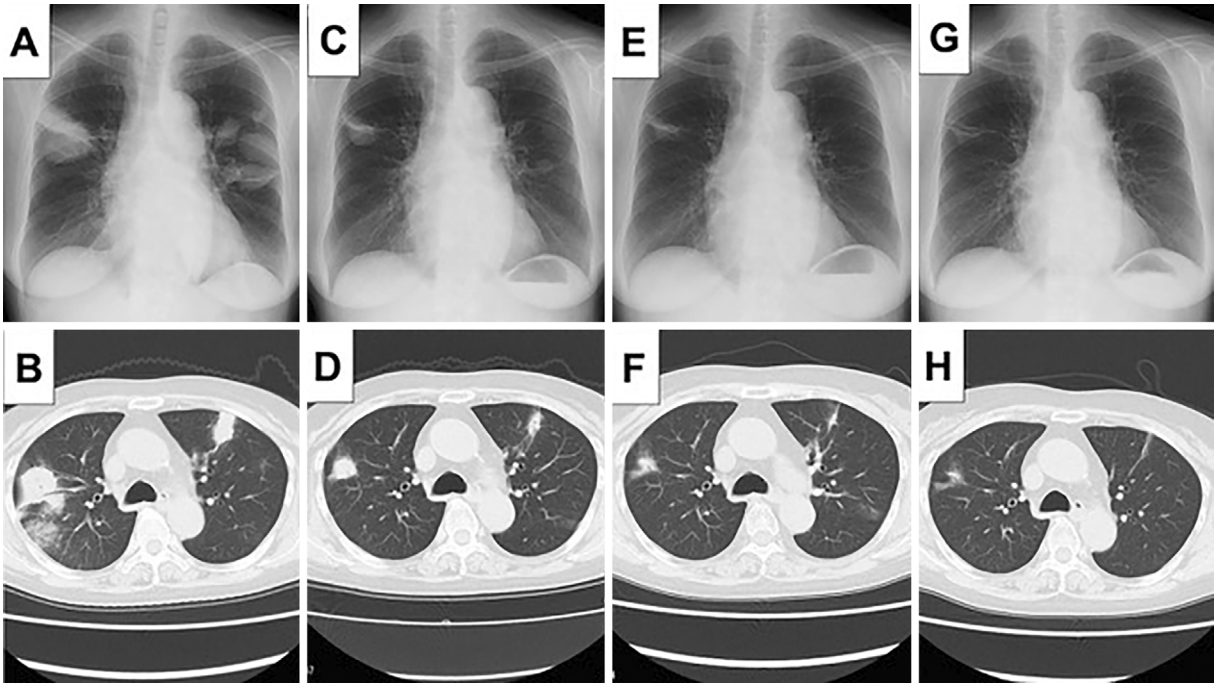

Figure. **The patient's chest radiography and computed tomography (CT) findings. (A) Chest radiography and (B) chest CT findings on admission; (C) chest radiography and (D) chest CT after three weeks of treatment with ampicillin/sulbactam (AMPC/SBT); (E) chest radiography and (F) chest CT after three weeks of treatment with sulfamethoxazole/trimethoprim (ST); (G) chest radiography and (H) chest CT after nine weeks of treatment with ST. Multiple masses and nodules are seen predominantly in the upper lung fields and upper lobes, respectively, demonstrating a rim of ground-glass opacities on admission (A, B). After three weeks of treatment with AMPC/SBT, the masses and nodules had shrunk (C, D). The size of the nodules and masses reduced progressively, as seen via imaging examinations performed at three (E, F) and nine (G, H) weeks after ST initiation.**

Table 1. **Laboratory Findings of the Patient on Admission.**

Hematological parameters			Serological and biochemical parameters		
White blood cells	9,800 cells/μL	T-bil	0.70 mg/dL	T-SPOT®. TB	(−)
Neutrophil	92.8 %	AST	36.0 IU/L	Anti-MAC antibody	<0.5 IU/mL
Lymphocyte	3.9 %	ALT	32.0 IU/L	CEA	2.7 ng/mL
Basophil	0.1 %	LDH	461 IU/L	CYFRA	1.3 ng/mL
Eosinophil	0.1 %	TP	5.7 g/dL	Pro-GRP	44.4 pg/mL
Monocyte	3.1 %	Alb	2.9 g/dL	sIL-2R	1,850 IU/mL
Red blood cells	406×10^4 cells/μL	BUN	15.0 mg/dL	CMV pp65 antigenemia	(+)
Hemoglobin	11.9 g/dL	Cr	0.91 mg/dL	1→3, β-D glucan	(−)
Hematocrit	35.6 %	CRP	15.1 mg/dL		

Laboratory tests revealed elevated leukocyte and neutrophil counts and elevated levels of CRP, AST, and LDH. The CMV antigen was positive.

ALT: alanine transferase, AST: aspartate aminotransferase, BUN: blood urea nitrogen, CEA: carcinoembryonic antigen, CMV: cytomegalovirus, Cr: creatinine, CRP: C-reactive protein, CYFRA: cytokeratin 19 fragment, LDH: lactate dehydrogenase, MAC: *Mycobacterium avium* complex, Pro-GRP: pro-gastrin-releasing-peptide, sIL-2R: soluble interleukin-2 receptor, T-bil: total bilirubin, TB: tuberculosis, TP: total protein

laxis with oral ST (80 mg trimethoprim/400 mg sulfamethoxazole on alternate days). Chest radiography revealed multiple bilateral pulmonary nodules and consolidations that had not been present two months prior to the follow-up consultation (Figure A). Computed tomography (CT) revealed relatively well-defined nodules and cavitary masses in both lungs, surrounded by ground-glass opacities, which were located predominantly in the upper lobes (Figure B). Laboratory examinations showed an increase in the neutrophil count and C-reactive protein level and a positive cytomegalovirus (CMV) pp65 antigenemia assay; however, her 1→3,β-D glucan level was within normal limits (Table 1).

Given the patient's immunocompromised state, she was

admitted to the hospital immediately. Under suspicion of a bacterial or cytomegalovirus infection, she was placed simultaneously on oral ampicillin/sulbactam (AMPC/SBT; 750 mg/day) and valganciclovir (900 mg/day), respectively. Bronchoscopy was performed, and bronchoalveolar lavage culture of a specimen from the right B3 bronchus using sheep blood agar and chocolate agar revealed *Nocardia* sp. No other bacteria, fungi, or mycobacteria were isolated. A transbronchial biopsy of the right B3aii and B2bii was also performed. Although periodic acid-Schiff-positive microorganisms were not observed clearly, fine Gram-positive organisms with a branching structure were observed, suggesting the possibility of *Nocardia*. A pathological examination did not reveal any findings suggestive of viral infection. The patient became afebrile and was discharged on hospital day 9. We used nanopore sequencing (MinION™ FLO-MIN107 R9.5 Version; Oxford Nanopore Technologies, Oxford, UK) technology and 16S rDNA capillary sequencing (ABI PRISM 3130 and BigDye Terminator Cycle Sequencing Ready Reaction Kit, PE Applied Biosystems, Foster City, USA) to identify the species (Supplementary material). The nanopore and capillary sequence results confirmed *N. elegans* [mapped reads: 89.8%, homology: 1,462/1,462 (100%)]. Brain magnetic resonance imaging revealed no findings suggestive of brain nocardiosis.

Based on the antimicrobial susceptibility test results (Supplementary material), AMPC/SBT was discontinued, and ST (treatment dose; 15 mg/kg trimethoprim), which is a more appropriate treatment for nocardiosis, was initiated. Eighteen days after treatment initiation, she developed neutropenia, which was considered an adverse effect of ST. As a result, ST was discontinued for 1 week and reinitiated at half of the initial dose (7.5 mg/kg trimethoprim) after neutropenia improved. Since then, her white blood cell count has been maintained at >2,800 cells/μL. Her serum CMV pp65 antigenemia was negative after 11 weeks of valganciclovir therapy. Follow-up CT performed three and nine weeks after ST initiation revealed the gradual improvement in pulmonary shadows (Figure F-H). ST treatment was completed 12 months after its initiation, and no recurrence of abnormal pulmonary shadows has been noted since then.

Discussion

We encountered a case of pulmonary *N. elegans* infection in a patient who was receiving ST as prophylaxis for PCP after renal transplantation. Although the epidemiology of *Nocardia* sp. infection, which is pathogenic to humans, varies with the geographic region and generation, the *N. asteroides* complex that includes *N. farcinica* and *N. nova* is frequently isolated in pulmonary nocardiosis (8-10). *N. elegans,* which belongs to the *N. vaccinii* clade, is a rare species isolated in nocardial infections; only 14 cases have been reported previously (Table 2) (11-23). Relevant studies, including our own, have included a higher proportion of men than women (8/14; the sex of 2 patients was unknown), with a mean patient age of 52.0±19.5 (range: 12-73) years old. The most frequent infection site was the lungs (10/14), and only two cases of central nervous system infection were reported. The cases occurred mainly in East Asian countries, including Japan, South Korea, China, and Taiwan. Only our patient and the patient described by Park et al. (13) had been receiving ST as PCP prophylaxis after renal transplantation prior to the diagnosis. The patient described by Ueda et al. (18) had been receiving prednisolone for systemic lupus erythematosus.

The recently reported incidence of nocardial infection after renal transplantation was 0.4-1.3%, of which 65.2% were pulmonary nocardiosis cases (24). To our knowledge, only one previous case of *N. elegans* infection has been reported in a patient after renal transplantation. The Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines recommend that all kidney transplant recipients receive ST as PCP prophylaxis after transplantation (25); however, some cases of nocardial infection have been reported, despite the administration of prophylactic ST. In a previous report, ST resistance was related to the onset of breakthrough nocardiosis (13); however, our patient did not present with ST resistance, which suggests that the prophylactic dose of ST could not prevent the onset of breakthrough nocardiosis completely due to the presence of multiple risk factors in the host.

Furthermore, in another previous study of allogeneic hematopoietic stem cell transplant recipients, the incidence of nocardiosis increased after ST was replaced by atovaquone for PCP prophylaxis (26). Currently, ST is the most suitable prophylactic drug for nocardiosis after renal transplantation.

In addition, an ST treatment course of at least six months is recommended, even for immunocompetent patients (27). Our patient had a relatively good response to the therapeutic dose of ST. Therefore, the treatment was continued for 12 months, considering the patient's immunocompromised state.

Nocardia has traditionally been classified morphologically, but the identification of specific species is a difficult task requiring specialized knowledge. The 16S rRNA method, which is conservative and species-specific, is often used for accurate taxonomic identification (28). Although capillary sequencing is the primary method for genetic characterization of *Nocardia* spp., to our knowledge, this is the first time that nanopore sequencing has been successfully used to identify *N. elegans* in a clinical isolate. So far, this technology has also been used for specific tasks, including Zika virus identification (5), identification of liver abscess-causing bacteria (6), and detection of human papillomaviruses in the cervix microbiome (7). Nanopore sequencing is a simple method that may contribute to the rapid identification of bacterial species in clinical practice, even in the identification of rare species. In addition, because nanopore sequencing is used for antimicrobial resistance gene detection (29), it may be used for drug resistance screening.

Several limitations associated with the present study warrant mention. First, the effectiveness of nanopore sequencing

Table 2. Clinical Features and Outcomes of *Nocardia elegans* Infection in the Present Case and Previously Reported Cases.

No.	References	Site of isolate	Clinical manifestation/ site	Background	Prophylaxis with ST	Country	Treatment	Outcome
1	11	Sputum	Pulmonary infection	···	···	Germany	···	···
2	12	···	Bronchitis	···	···	Japan	···	···
3	13	Punctured pus	Lung abscess	Kidney transplantation	Y	Korea	ST+IMP/CS ⇒ AMPC/CVA	Improved
4	14	Sputum	Pulmonary infection	Cystic fibrosis	···	Spain	MEPM+TOB	Improved
5	15	···	Pulmonary infection	Dermatomyositis	N	Taiwan	···	···
6	16	Synovial fluid	Purulent arthritis	···	···	Japan	···	···
7	17	Sputum	Pulmonary infection	Still's disease	···	Japan	IMP/CS+AMK	Improved
8	18	Sputum	Pulmonary+brain	Systemic lupus erythematosus	Y	Japan	ST+CAM	Improved
9	19	Skin abscesses and vitreous fluid	Pulmonary+eye+skin	Rheumatoid arthritis	N	Japan	CFPM ⇒ ST ⇒ IMP/CS+MINO	Survived but lost vision+ resistant
10	20	Sputum and skin lesion	Pulmonary+skin	Diabetes mellitus	N	China	Penicillin+DOXY+ST	Improved
11	21	Punctured pus and central nervous system	Pulmonary+renal+brain	Renal tumour	···	Mali/ France	PIPC/TAZ+AMK ⇒ IMP/CS+CPFX	Died+ resistant
12	22	Bronchial lavage	Pulmonary	Systemic lupus erythematosus	…	Japan	ST	Improved
13	23	Punctured pus	Pulmonary+subcostal muscle	Hematopoietic stem cell transplantation	N	Japan	CAM	Improved
14	Present case	Transbronchial lung biopsy	Pulmonary	Kidney transplantation	Y	Japan	ABPC/SBT ⇒ ST	Improved

ABPC/SBT: ampicillin/sulbactam, AMK: amikacin, AMPC/CVA: amoxicillin/clavulanate, CAM: clarithromycin, CFPM: cefepime, CPFX: ciprofloxacin, DOXY: doxycycline, IMP/CS: imipenem/cilastatin, MEPM: meropenem, MINO: minocycline, N: no, PIPC/TAZ: piperacillin/tazobactam, ST: sulphamethoxazole/trimethoprim, Y: yes

in gene extraction is limited. The accuracy may be improved by the simultaneous use of 16S rRNA amplification or by increasing the amount of nucleic acid extracted from bacterial samples. Second, a bacterial co-infection could not be excluded because empirical treatment with AMPC/SBT was administered. However, our patient was immunocompromised after renal transplantation and needed empiric antimicrobial therapy. Third, CMV pneumonia and antiviral treatment may have affected the progression of pulmonary shadows. However, while bronchoscopy detected *Nocardia* in the area of abnormal shadows, no microscopic morphological changes suggestive of a viral infection were observed. Therefore, we believe that our patient mainly had a nocardial infection.

Conclusion

We herein report a rare case of pulmonary infection caused by *N. elegans* in a patient who was immunosuppressed after undergoing renal transplantation. The bacterial species was rapidly identified using nanopore sequencing. It is important to note that *Nocardia* may infect patients receiving ST, even though ST is considered the most effective prophylactic drug.

This study was approved by the National Defense Medical College Review Committee.

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent is available for review.

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

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