Tsukamurella pulmonis central venous catheter infection mimicking proteinase 3-antineutrophil cytoplasmic antibody (PR3-ANCA)-associated vasculitis

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ABSTRACT
A 40-year-old Japanese woman, who underwent total thyroidectomy, had suffered from repeated episodes of fever and microscopic hematuria for 3 years, which had started 3 months after central venous port catheter insertion. On admission, she had malaise and low-grade fever, and was found to have microscopic hematuria, urinary red blood cell casts, multiple pulmonary nodules, and positivity of proteinase 3-anti-neutrophil cytoplasmic antibody (PR3-ANCA), which were suggestive to the presence of ANCA-associated small vessel vasculitis. However, her blood culture and subsequent gene analysis revealed the positivity of Tsukamurella pulmonis, and she was diagnosed with Tsukamurella pulmonis bacteremia accompanying PR3-ANCA positivity. Her condition improved after the removal of the catheter and antibiotic treatment. Tsukamurella species are categorized to the order Actinomycetales and can be misidentified as other Actinomycetales without genetic analyses. This case illustrates that chronic Tsukamurella pulmonis infection can cause ANCA production and nephritis, which mimics ANCA-associated vasculitis. Thus, it is critical to diagnose these cases correctly to avoid misdiagnosis and inappropriate treatment, such as immunosuppressive treatment.

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1. Introduction
Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of diseases, such as granulomatosis with polyangiitis, microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis, characterized by destruction and inflammation of small vessels [1,2]. AAV can affect several organs, including the kidney and lung [1,2]. Chronic microbial infections, such as infectious endocarditis, have been reported to show ANCA positivity, by triggering the production of ANCA [1,2]. Clinical features of such chronic infections sometimes resemble AAV, which can lead to misdiagnosis and inappropriate treatment, including immunosuppressive therapy. Thus, a precise diagnosis is required to avoid such misdiagnosis and possible life-threatening outcomes. We herein describe a case of chronic venous catheter infection by Tsukamurella pulmonis which mimicked proteinase-3 (PR3)-ANCA-associated vasculitis. Chronic Tsukamurella pulmonis infection has not previously been reported as a potential trigger for ANCA positivity and subsequent nephritis.

2. Case presentation
A 40-year-old Japanese woman had a central venous (CV) port catheter inserted in her right subclavian vein for intravenous calcium supplementation because of treatment-resistant tetany after undergoing total thyroidectomy for hyperparathyroidism. Three months after the implantation of the CV port catheter, she developed a low-grade fever accompanied by elevation of serum C-reactive protein levels and microscopic hematuria. She was treated with levofloxacin following a provisional diagnosis of pyelonephritis and improved within days. She had experienced repeated similar febrile episodes, which responded well to levofloxacin, approximately every two months for three years. Blood cultures had been performed several times previously, and had shown negative results. Two months before the admission, she had presented with malaise, low-grade fever,
and respiratory discomfort. At that time, her chest computed tomography (CT) examination revealed multiple nodules in both the lungs (Figure 1). She was diagnosed with pneumonia and treated with levofloxacin. Despite the administration of levofloxacin, her symptoms had gradually deteriorated, leading to her admission to our hospital.

On admission, a physical examination revealed a body temperature of 37.3°C, blood pressure of 121/77 mmHg, a pulse rate of 93 beats/min, and oxygen saturation (SpO2) of 97% in room air. She had no skin abnormalities, including purpura, Osler's nodes, and Janeway lesions, and there were no inflammatory skin changes around the implanted CV port. Laboratory tests revealed a white blood cell count of 8000/μL, hemoglobin level of 11.8 g/dL, platelet count of 31.4 × 10^5/μL, blood urea nitrogen level of 9.5 mg/dL, serum creatinine level of 0.62 mg/dL, and C-reactive protein (CRP) level of 2.25 mg/dL (Table 1). Urinalysis revealed 1+ proteinuria and 2+ occult hematuria. Urinary sedimentation examination revealed red blood cells (RBC) 30–40/high power field (>50% dysmorphic) and RBC casts 20–29/whole field. Immunological analyses revealed the positivity of PR3-ANCA (38.8 U/mL). Myeloperoxidase (MPO)-ANCA was negative. Transthoracic echocardiography revealed no valvular abnormalities and no vegetation. Abdominal CT examination revealed no stones and deformities of the kidneys. Blood culture examination revealed the presence of gram-positive rods (Figure 2(A)), which were detected in the 115-hour culture of an arterial blood sample and the 41-hour culture of a blood sample drawn through the CV port catheter. Further bacterial examination revealed that the bacteria were negative on Ziehl-Neelsen staining (Figure 2(B)) and positive on Kinyoun staining (Figure 2(C)), indicating a weak acid resistance. Gene sequencing analysis for 16S ribosomal RNA identified the bacteria as Tsukamurella pulmonis.

Based on these findings, she was diagnosed with Tsukamurella pulmonis bacteremia due to catheter infection. Subsequently, her CV port catheter was removed, and she was treated with rifampicin (RFP; 300 mg/day), teicoplanin (TEIC; 400 mg/day), and vancomycin (VCM; 1 g/12 h) (Figure 3). Seeing as there is no standard treatment for Tsukamurella species infections, we started treatment with RFP, TEIC, and VCM based on a publication on catheter-related bloodstream infections caused by Tsukamurella [3]. Her clinical symptoms improved over a period of several days following treatment. The antibiotics were continued for eight weeks. Her CRP returned to normal in a month, her urinary abnormalities resolved, and her pulmonary nodules disappeared. Further, her PR3-ANCA levels decreased to within the normal range during the following year (Figure 3). She was observed for two years, without recurrent episodes of fever and urinary abnormalities.

Figure 1. Chest computed tomographic images. Multiple pulmonary nodules with surrounding ground-glass opacities (Arrow heads) are visible in both lungs.

Table 1. Results of the laboratory tests conducted on admission.

| Hematology          | Serology        |
|---------------------|-----------------|
| WBC 8000/μL         | CRP 2.25 mg/dL  |
| Neut 71%            | ANA <40         |
| Eo 1%               | C3 143 mg/dL    |
| Ba 0%               | C4 29 mg/dL     |
| Ly 23%              | CH50 42.5 IU/mL |
| Mono 5%             | MPO-ANCA <1.0 IU/mL |
| RBC 4.66 × 10^6/μL  | PR3-ANCA 37.3 IU/mL |
| Hb 11.8 g/dL        | RF 87 U/mL      |
| Hct 36.4%           |                 |
| Plt 31.4 × 10^5/μL  | Urinalysis      |
|                    | Protein 1+      |
| Biochemistry        |                 |
| TP 7.7 g/dL         | Glucose –       |
| Alb 3.9 g/dL        | Occult blood 2+ |
| ALT 11 U/L          | Leukocyte 1–4/HPF |
| AST 18 U/L          | RBC 30–40/HPF  |
| LDH 230 U/L         | RBC cast 20–29/WHF |
| BUN 9.5 mg/dL       | Bacteria 1+     |
| Cre 0.62 mg/dL      |                 |
| UA 5.0 mg/dL        |                 |
| Na 144 mmol/L       |                 |
| K 4.1 mmol/L        |                 |
| Cl 106 mmol/L       |                 |
| Ca 7.1 mg/dL        |                 |
3. Discussion

This case demonstrates three unique points: 1) Tsukamurella pulmonis causes chronic catheter infection in a non-immunocompromised individual; 2) the formation of ANCA is induced by chronic Tsukamurella pulmonis infection; and 3) Tsukamurella bloodstream infection mimics ANCA-associated vasculitis.

Tsukamurella species are aerobic gram-positive rods that are found in a broad range of environments such as soil, water, and sludge [3,4]. Tsukamurella species are categorized to the order Actinomycetales and have many features similar to other Actinomycetales, such as Nocardia, Rhodococcus, Gordonia, and rapidly growing Mycobacterium bacteria [3]. Hence, Tsukamurella species can be misidentified as one of these genera using standard microbiological tests [3]. However, the identification of Tsukamurella can be achieved by 16S ribosomal RNA gene sequencing [4,5]. Clinical features of Tsukamurella infection include catheter-related bloodstream infections, skin and soft tissue infections, respiratory tract infections, conjunctivitis, and brain abscesses [3,4,6]. In the present case, Tsukamurella infection developed as a catheter-related bloodstream infection. Although most reported cases of Tsukamurella bacteremia were limited to immunocompromised patients [3,6–8], our patient was a non-immunocompromised individual. This suggests that Tsukamurella infection can occur not only in immunocompromised individuals but also in immunocompetent individuals on a specific occasion, such as intravenous catheter insertion. Since Tsukamurella species have been misdiagnosed as other Actinomycetales in the absence of correct molecular diagnostic methods [4,5], the importance of Tsukamurella species in disease presentation and pathogenesis may have been underestimated in clinical settings. Therefore, appropriate diagnosis and accumulation of clinical data are required to develop proper therapeutic strategies for Tsukamurella infection.

The formation of ANCA in our patient is likely to have been induced by a chronic Tsukamurella pulmonis infection. ANCA formation is induced during chronic infections of various viral, bacterial, fungal, and multicellular parasitic etiology [9–11]. Infectious endocarditis and tuberculosis are the typical infectious causes of ANCA formation. For example, it has been reported that 20 (18%) out of 109 patients with infectious endocarditis had ANCA detected by indirect immunofluorescence (IIF), and 8% had PR3-ANCA or MPO-ANCA detected by enzyme-linked immunosorbent assay (ELISA) [12]. Also, in 45 patients with tuberculosis, ANCA was detected in 20 patients (44.4%) by IIF and in 18 patients (40%) by ELISA (15 PR3-ANCA, 3 MPO-ANCA) [13]. In terms of the mechanisms, neutrophil extracellular traps (NETs) have been reported to be involved in the process of ANCA production [10,14,15]. Neutrophils stimulated during infection form NETs, which contain histones, PR3, and MPO, all of which are antibacterial proteins. Prolonged exposure to their contents disrupts tolerance to PR3 and MPO, resulting in the production of ANCA [10,14]. Several drugs are reported to induce ANCA formation, which include propylthiouracil, hydralazine, and montelukast [16]. Our patient had been treated with quetiapine fumarate, aripiprazole, and

Figure 2. Staining images of the isolated bacteria from blood culture. Gram-positive rods are present on Gram staining (A); the bacteria are negative in Ziehl-Neelsen staining (B); and positive in Kinyoun staining (C).
mirabegron by a psychiatrist and a urologist. The drugs have been continued throughout the clinical course. No drugs which are reported to induce ANCA formation had not been applied to the patient. Thus, drug-induced ANCA formation is less likely in the present case.

Since our patient exhibited urinary abnormalities and multiple pulmonary nodules, we initially suspected the presence of small-vessel vasculitis associated with PR3-ANCA. However, because Tsukamurella pulmonis was detected during blood testing, we realized that her urinary and pulmonary findings were caused by Tsukamurella infection. Hence, the clinical findings were presumed to be caused by infection-related glomerulonephritis, with pulmonary foci of secondary infection caused by the bloodstream infection. This diagnosis was supported by her favorable clinical course without fever and urinary abnormalities after the administration of antibiotics. In retrospect, repeated episodes of fever and microhematuria are presumed to be caused by Tsukamurella bloodstream infection and the infection-related glomerulonephritis.

In conclusion, Tsukamurella pulmonis can cause chronic catheter infection in immunocompetent individuals, which induces ANCA production and nephritis, mimicking AAV. As this case demonstrates, clinicians should be aware that AAV and ANCA-positive chronic infections have substantial similarities in both clinical and laboratory manifestations. It is critical to diagnose these cases correctly to avoid misdiagnosis and inappropriate treatment, such as immunosuppressive treatment.

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Informed consent
Patient consent was received to publish this case report, with the knowledge that the patient’s identity is fully anonymized and that they cannot be identified from this case report.

Disclosure statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figure 3. Clinical course of the patient. Her central venous (CV) port catheter was removed, and she was treated with rifampicin (RFP), teicoplanin (TEIC), and vancomycin (VCM). Her symptoms resolved following treatment. Her serum C-reactive protein (CRP) levels decreased to within the normal range within a month, and proteinase-3-antineutrophil cytoplasmic antibody (PR3-ANCA) levels returned to within the normal range within a year.
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