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Mycotic Aortic Aneurysm Associated with *Legionella anisa*▽

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***Legionella anisa* is rarely associated with human disease. Its gene was identified by broad-range PCR in whole blood and excised tissue from a patient with a culture-negative mycotic aneurysm and was considered as a possible pathogen. This case report is potentially useful for the future diagnosis of intravascular infection.**

CASE REPORT

The patient was a 79-year-old healthy man with a history of Y-graft replacement for an abdominal aortic aneurysm 3 years ago. Although the postoperative treatment course had been uneventful, he complained of high fever and tenderness of the right inguinal region 1 week before admission. After two sets of blood cultures were drawn, levofloxacin at 200 mg orally (p.o.) every 12 h was prescribed at the outpatient clinic 4 days before admission. However, his condition worsened and he was subsequently admitted to our hospital. Upon admission, his lungs were clear to auscultation and a vascular murmur and tenderness were observed in the right inguinal region. A chest X-ray showed no infiltrate. Laboratory data revealed a leukocyte count of 9,130/mm^3 and an elevated C-reactive protein level of 20.1 mg/dl. A BinaxNOW *Legionella pneumophila* urinary antigen test was negative. Blood samples were cultured with the BacT/Alert 3D blood culture system (bioMérieux) by using both aerobic and anaerobic media (11). Blood cultures collected at the outpatient clinic and upon admission showed negative results. We stopped levofloxacin upon admission and repeated blood cultures 2, 3, and 4 days after cessation of the antibiotic. However, the blood cultures were all negative. Enhanced computed tomography (CT) revealed a pseudoaneurysm at the anastomotic site of the artificial vessel and the right common iliac artery (Fig. 1A), and ^{67}Ga scintigraphy showed abnormal uptake at the same site (Fig. 1B), suggesting a mycotic aortic aneurysm. Vancomycin at 1 g intravenously (i.v.) every day and meropenem at 0.5 g i.v. every 12 h were started empirically for a culture-negative mycotic aneurysm on the fifth day after admission.

A broad-range PCR targeting the bacterial 16S rRNA gene, followed by direct sequencing, was performed on whole blood to obtain additional information. Bacterial DNA was extracted by the phenol-chloroform-isoamyl alcohol procedure with a MORA-EXTRA kit (Kyokuto Pharmaceutical, Tokyo, Japan) (9). PCR primers were designed to detect two of the conserved regions of the 16S rRNA gene (Table 1). PCR was performed on a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA). DNA was amplified as follows: 10 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 57°C, and 30 s at 72°C; and finally 10 min at 72°C. Amplified products were sequenced and compared with known bacterial gene sequences by using BLAST (available at the National Center for Biotechnology Information [http://www.ncbi.nih.gov/BLAST/]) (13, 16). As a result, *Legionella anisa* was suspected on the basis of ≥98% BLAST similarity. To confirm this result, we performed an additional assay that targeted *Legionella*-specific regions within the macrophage inhibitor potentiator (*mip*) gene (5). DNA was amplified as follows: 10 min at 95°C; 30 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C; and finally 10 min at 72°C. The *mip* gene-based PCR was positive, and *L. anisa* was identified (Table 1).

Although clarithromycin at 200 mg p.o. every 12 h was added to the i.v. administration of vancomycin and meropenem, subsequent CT demonstrated an increase in the size of the aneurysm. An urgent operation, including resection of the infected aneurysm and replacement of the aortic graft, was performed on the 11th day after admission (Fig. 2). No organisms were identified by Gram staining and Gimenez staining of the excised aortic wall tissue. Cultures performed with blood agar plates with 5% sheep blood and BCYE-agar plates were negative (5). PCRs targeting the 16S rRNA gene and the *mip* gene were performed with the excised tissue and identified *L. anisa* again. As the *L. anisa* gene was detected in both preoperative whole blood and excised tissue, this was considered as a possible pathogen responsible for this mycotic aneurysm. After surgery, linezolid at 600 mg i.v. every 12 h for 2 weeks and pazufloxacin at 500 mg i.v. every 12 h for 3 weeks were administered. The postoperative course was uneventful, and i.v. antibiotics were followed by levofloxacin at 300 mg p.o. every 12 h and clarithromycin at 200 mg p.o. every 12 h.

Mycotic aortic aneurysms are difficult to treat and are associated with significant mortality. Successful resolution

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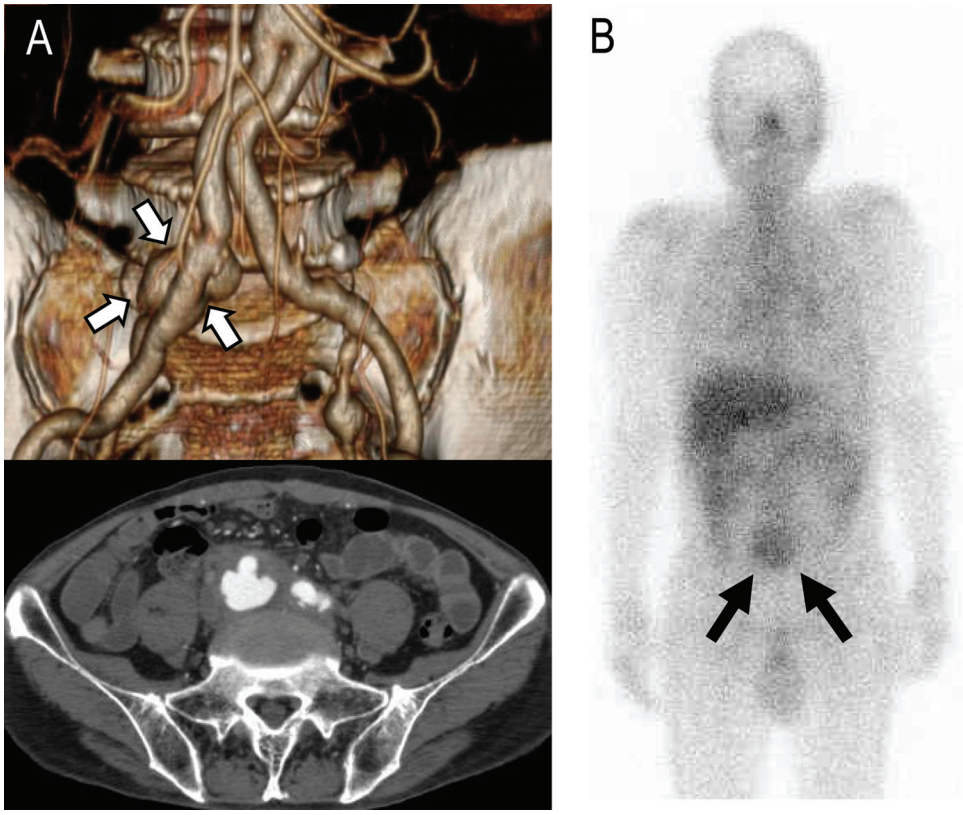

FIG. 1. (A) Enhanced CT upon admission showing a pseudoaneurysm (arrows) at the anastomotic site of the artificial vessel and the right common iliac artery. (B) ^{67}Ga scintigraphy showing abnormal uptake (arrows) at the same site as the pseudoaneurysm.

TABLE 1. Primer sequences used for broad-range bacterial and *Legionella*-specific PCRs and sequences of amplified products

Application	Primer name, sequence	Product size (bp)	Sequence of amplified product
16S rRNA gene PCR	Uni-F (forward), 5′-CCAGCAGCCGCGGTAATAC Uni-R (reverse), 5′-CCCCGTCAATTCCTTTGAGTT	400	GGTAATACGGAGGGTGCAAGCGTTAATCGGA ATTACTGGGCGTAAAGCGTGCGTAGGTGGT TGATTAAGTTATCTGTGAAATCCCTGGGCTT AACCTGGGCAGGTCAGATGATACTGGTTGA CTCGAGTATGGGAGAGGGTAGTGGAATTTC CGGTGTAGCGGTGAAATGCGTAGAGATCG GAAGGAACACCAGTGGCGAAGGCGGCTAC CTGGCCTAATACTGACACTGAGGCACGAAA GCGTGGGGAGCAAACAGGATTAGATACCC TGGTAGTCCACGCTGTAAACGATGTCAACT AGCTGTTGGTTATATGAAAATAATTAGTGG CGCAGCAAACGCGATAAGTTGACCGCCTGG GGAGTACGGTCGCAAGATTAAAACTCAAAG GAATTGACGGG
mip PCR	Mip-F (forward), 5′-TGGCAATGTCTACTGTAATGG Mip-R (reverse), 5′-ACAGTTACTGTGTCCGCTTTACC	406	CTGTAATGGCAGCTGATGCTACATCGCTTGTT ACGGATAAGGATAAATTATCTTATAGTATT GGTGCTGATTTAGGAAAAAATTTCAAAAAT CAAGGTATTGATATTAATCCGGACGCATTA GCTAAAGGAATGCAAGACGGAATGTCTGG TGCCCAATTGATTTTGACAGAACAACAAAT GAAAGATGTTCTGAATAAATTCCAAAAAGA GTTGATGGCGAAACGCAGCGCTGAGTTTAA TAAAAAAGCTGAAGAAAACAAATCTAAAG GCGATGCTTTTTTATCAACTAACAAATCAAA ATCTGGCGTAACGGTACTGCCAAGCGGTTT ACAATATAAAGTTATTGAAGCAGGTACAGG AAATAAACCCGGTAAAGCGGACACA

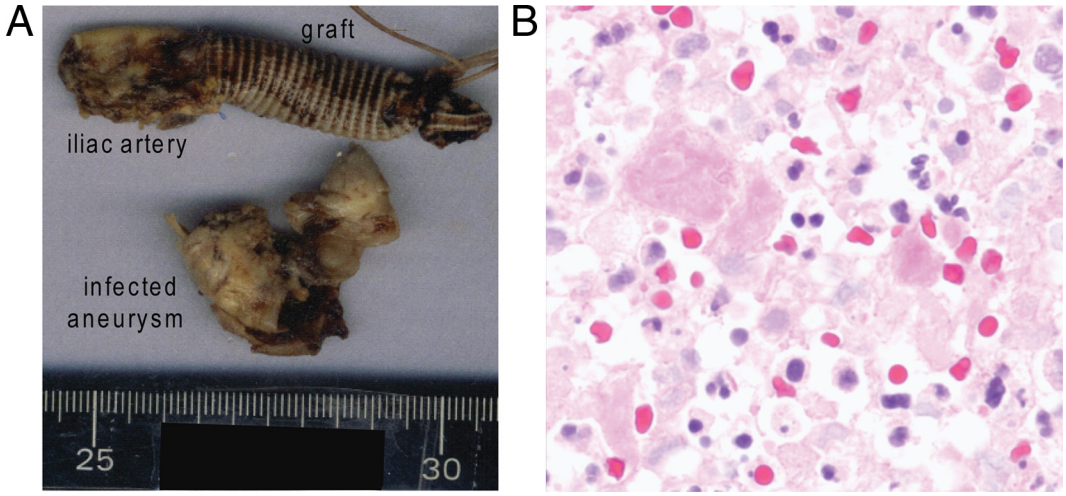

FIG. 2. (A) Excised aortic graft attached to the iliac artery and infected aneurysm (dimensions, 2.0 by 2.5 cm). (B) Pathological tissue sample stained with hematoxylin and eosin showing infiltration of inflammatory cells including neutrophils and atherosclerotic change.

of infected aneurysms depends on early diagnosis, prolonged systemic antibiotic therapy, and timely surgical intervention (8). Intensive antibiotic therapy is crucial for successful treatment. A broad-spectrum antibiotic should be used until culture sensitivity reports are available and a specific antibiotic is determined (12). Therefore, identification of the causative microorganism is important. The organisms most commonly found to be responsible for mycotic aneurysms are *Salmonella* species and *Staphylococcus aureus*, but cultures are negative in some cases (8, 12).

Legionella bacteria are small, gram-negative bacilli with fastidious growth requirements. More than 49 different *Legionella* species have been described, and 20 have been reported to infect humans (6). Although *L. pneumophila* accounts for most clinical cases, there are some case reports in which *L. anisa* has been isolated from patients (1, 15). *Legionella* infections are caused by the inhalation of aerosols generated from water sources contaminated with *Legionella* bacteria and usually result in pneumonia. Extrapulmonary infections are rare and usually occur as metastatic complications of pneumonia in immunocompromised patients. Although cardiac muscles, the pericardium, and vascular shunts and grafts have been reported as extrapulmonary cardiovascular *Legionella* infection sites, as far as we know, this is the first case of a mycotic aortic aneurysm associated with *L. anisa* (6).

The patient had no history of visiting hot springs, and there is no 24-h hot spring bath system in his house, but he has a big pond and a well in his garden. We ordered the testing of water from his garden for the presence of *Legionella* bacteria by an outside laboratory. No *Legionella* species was cultured by selective Wadowsky-Yee-Okuda agar medium (10). The source of the *L. anisa* infection remains unknown.

Diagnostic tests for Legionnaires' disease include cultures, urine antigen testing (only for *L. pneumophila*), immunofluorescence microscopy, antibody testing with paired sera, and molecular amplification (6, 7). Although culture tests are the "gold standard" for the diagnosis of Legionnaires' disease, many clinical laboratories lack the expertise required for testing. In this case, preoperative blood culture and excised tissue cultures, including the use of BCYE-agar plates, were negative. Alternatively, we identified *L. anisa* gene in his blood and excised tissue by PCR and found it to be the possible pathogen responsible for this patient's mycotic aortic aneurysm. Molecular techniques combined with broad-range PCR amplification and direct sequencing have been useful tools to diagnose culture-negative cases involving pathogens that are difficult to culture or cases involving prior antibiotic treatment (2–4). Our group has applied this technique to culture-negative intravascular infection cases and also reported a case of culture-negative infective endocarditis (14). When using molecular techniques, care must be taken to consider the possibility of false positives due to contamination or transient bacteremia, especially when working with whole-blood samples (4).

We routinely perform PCRs for culture-negative cases, and this is the only case in which the *L. anisa* 16S rRNA gene was detected. As the *L. anisa* gene was detected at different times and places in this patient, we believe that the possibility of extraneous contamination is very low. We believe that the *L. anisa* gene came from the patient's blood and tissue; however, we could not decide whether this organism really caused the patient's mycotic aneurysm or only colonized him because *L. anisa* is a very low-virulence pathogen.

In conclusion, we encountered a case of mycotic aortic aneurysm with detection of the *L. anisa* gene by broad-range PCR. This alternative technique may decrease the number of undiagnosed culture-negative cases and may be useful for selecting appropriate antibiotics.

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