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Prosthetic Valve Endocarditis and Bloodstream Infection Due to *Mycobacterium chimaera*

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Prosthetic valve endocarditis (PVE) due to fast-growing nontuberculous mycobacteria (NTM) has been reported anecdotally. Reports of PVE with slowly growing NTM, however, are lacking. We present here one case of PVE and one case of bloodstream infection caused by *Mycobacterium chimaera*. Randomly amplified polymorphic DNA (RAPD)-PCR indicated a relatedness of the two *M. chimaera* strains. Both patients had heart surgery 2 years apart from each other. A nosocomial link was not detected.

Infective endocarditis due to nontuberculous mycobacteria (NTM) is a rare complication after heart valve surgery. The reported cases in the literature are associated with the insertion of biological and also of mechanical valves (1). Cases of prosthetic valve endocarditis (PVE) due to NTM often involve rapidly growing mycobacteria. To date, there have been no concise reports on slowly growing mycobacteria, such as the *Mycobacterium avium* complex (MAC), as the agent causing PVE. However, the MAC is found occasionally on resected heart valves. A case series examining microbiological cultures from valves found slowly growing NTM in 5.5% of the cases without further clinical or histopathological evidence of infective endocarditis (IE) (2).

MAC members are the most common cause of NTM infections in humans. *M. intracellulare* and *M. avium* are the main representatives of the MAC, but different MAC sequevars, e.g., *M. chimaera*, have been identified in recent years (3). Similar to other members of the MAC, *M. chimaera* has been reported to cause mainly pulmonary disease (3). In the summer of 2011, we encountered one fatal case of definite PVE and one fatal bloodstream infection due to *M. chimaera*. Randomly amplified polymorphic DNA (RAPD)-PCR was used to study the relatedness of the *M. chimaera* isolates from these two patients.

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CASE REPORTS

Patient 1. In June 2011, a 58-year-old male was admitted to the hospital for mitral and aortic valve replacement. In 2008, the patient had undergone aortic and mitral reconstruction with implantation of a mitral annuloplasty ring. Twelve months prior to the current admission, the patient experienced intermittent fever, weight loss, and respiratory distress. PVE was ruled out with repeated negative conventional blood cultures and a transesophageal echocardiogram that showed only moderate mitral and aortic insufficiency not suggestive of infective endocarditis. At that time, systemic sarcoidosis had been diagnosed based on unspecific granulomatous inflammation in liver and kidney biopsy specimens, a reticular pattern on the chest X ray together with a severely constrained CO diffusion capacity, and a bronchoalveolar lavage showing a predominance of lymphocytes but only a slightly elevated CD4/CD8 quotient. A *Mycobacterium* genus PCR from the preserved liver and kidney biopsy specimens was performed retrospectively 1 year later and showed negative results. Cultures from the bronchoalveolar lavage were negative for mycobacteria. Prednisone was given at a maintenance dose of 20 mg per day. Because of fatigue and edema in the patient's legs, the prednisone dose was increased to 50 mg in the interval. HIV testing (for HIV-1 and HIV-2) was negative. Despite immunosuppressive therapy with steroids, the general health condition of this patient deteriorated over the course of a year. In May 2011, the patient was hospitalized due to respiratory distress. Several conventional blood cultures remained negative, but a transesophageal echocardiogram showed evidence of severe mitral and aortic valve insufficiency. Therefore, the patient was referred to our tertiary care hospital for repeat valve surgery. On admission, the physical examination revealed pulmonary wheezing, a pansystolic heart murmur, and a chronic pilonidal sinus infection. Laboratory tests showed an elevated C-reactive protein level, and the patient was started on amoxicillin-clavulanic acid, assuming an infected pilonidal sinus. During surgery, a fraying of the prosthetic ring of the mitral valve was found, and there was evidence of destruction of the mitral and aortic cusps. Both valves were replaced by new bioprostheses. The antimicrobial therapy was changed to vancomycin and piperacillin-tazobactam in the setting of ventilator-associated pneumonia and intraoperative findings suggestive of infective endocarditis. The histopathological examination of the fibrous valve tissue showed a partly necrotizing, acute and chronic inflammation with numerous foamy macrophages containing many periodic acid-Schiff- and acid-fast-positive bacteria, consistent with the diagnosis of an acute necrotizing mycobacterial en-

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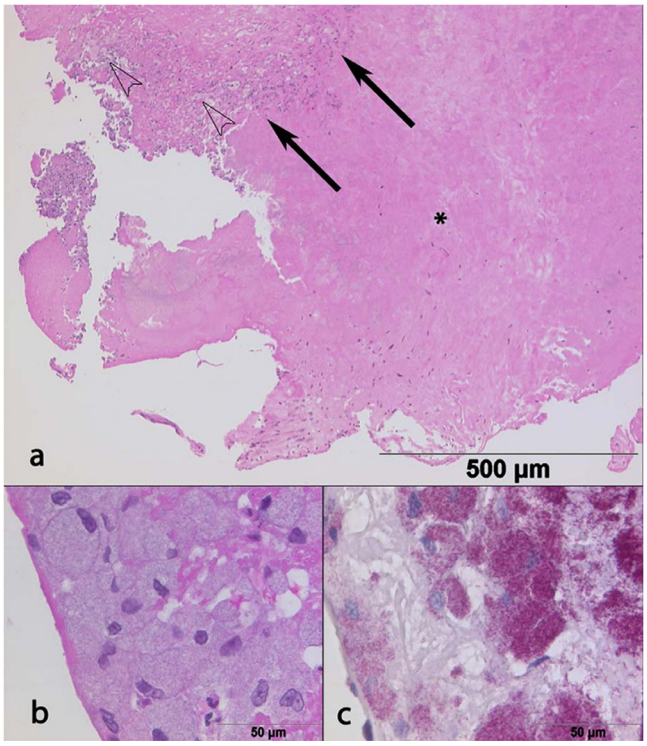

FIG 1 Histopathological analysis of valve tissue from patient 1. (a) Overview of the necrotic valve tissue (p) with granulocytic demarcation (arrows) and foamy macrophages (arrowheads) (hematoxylin and eosin [H&E] stain); (b) swollen foamy macrophages (H&E stain); (c) presence of numerous acid-fast bacilli (Ziehl-Neelsen stain).

docarditis (Fig. 1). Further molecular analysis of the formalin-fixed paraffin-embedded (FFPE) tissue showed the presence of *M. avium* complex (MAC). Subsequent cultures of the prosthetic ring of the mitral valve annulus allowed identification of *M. chimaera* based upon the 16S rRNA gene sequence. A disseminated NTM infection was confirmed by the molecular detection of *M. chimaera* in the tracheal secretion and three consecutive heparin blood cultures. Despite targeted drug therapy with clarithromycin, rifabutin, and ethambutol (Table 1), the patient died 15 days later due to progressive heart failure. Permission for an autopsy could not be obtained.

Patient 2. A 51-year-old man was hospitalized in July 2011 with a 4-month history of fever of unknown origin accompanied by splenomegaly, progressive renal insufficiency, elevated liver enzyme levels, and pancytopenia. His medical history was remarkable for a previous herpes zoster infection, candida esophagitis, and the insertion of a composite graft (a mechanical aortal valve and prosthetic aortic arch) after an aortal dissection in January 2010. During hospitalization, PVE was ruled out with transesophageal echocardiography. *M. chimaera* was cultivated from bone marrow samples, two different blood cultures, one urine sample, and a tracheal swab, prompting the diagnosis of a disseminated *M. chimaera* infection. The lymphocyte and CD4 cell counts were 380 and 237 cells/ml, respectively, suggesting a primary or secondary immune deficiency. A mutation in the interleukin 12 (IL-12)/interferon (IFN) pathway or an idiopathic CD4 lymphopenia was discussed as the underlying condition after an HIV infection had been excluded (by a screening antigen/antibody test, the HIV load, and a product-enhanced reverse transcriptase [PERT] assay). Targeted drug therapy with clarithromycin, rifabutin, and ethambutol was started at the end of August 2011 (Table 1). In April 2012, the patient died suddenly due to splenic rupture as a consequence of a splenic infarction. Coincidentally, the ophthalmologist ascertained multiple chorioretinal spots of various ages consistent with arterial emboli. The necropsy of the heart, the mechanical valve,

TABLE 1 Susceptibility testing of *M. chimaera* isolates from patients 1 and 2

Antimicrobial substance and dosages	Result for *M. chimaera* from patient 1[a]	Result for *M. chimaera* from patient 2[a]	
		Isolate 1[b]	Isolate 2[c]
Rifampin			
1 mg/liter	R	R	R
20 mg/liter	S	S	S
Rifabutin			
0.1 mg/liter	R	R	R
2 mg/liter	S	S	S
Amikacin			
1 mg/liter	R	R	R
4 mg/liter	I	I	I
20 mg/liter	S	S	S
Ofloxacin			
2 mg/liter	R	R	R
10 mg/liter	I	S	S
Moxifloxacin			
0.5 mg/liter	I	I	I
2.5 mg/liter	S	S	S
10 mg/liter	S	S	S
Clarithromycin			
4 mg/liter	S	S	S
16 mg/liter	S	S	S
32 mg/liter	S	S	S
64 mg/liter	S	S	S
Ethambutol			
5 mg/liter	S	S	S
50 mg/liter	S	S	S
Vancomycin			
4 mg/liter	R	R	R
8 mg/liter	R	R	R
16 mg/liter	R	R	R
32 mg/liter	R	R	R
Piperacillin/tazobactam			
4 mg/liter	R	R	R
8 mg/liter	R	R	R
16 mg/liter	R	R	R
32 mg/liter	I	R	R

[a] Note that the terms susceptible (S), intermediate (I), and resistant (R) describe *in vitro* growth inhibition at a given drug concentration and should not be confused with classifications according to clinical breakpoints intended to predict clinical outcome. The intermediate category indicates that the drug concentration examined significantly (. 99%), but not completely, inhibited bacterial growth *in vitro*.
[b] *M. chimaera* isolate obtained in the summer of 2011.
[c] *M. chimaera* isolate cultivated from urine postmortem in the spring of 2012.

and the arterial graft did not show any signs of endocarditis or a vascular graft infection. The postmortem analysis of the spleen, liver, and kidneys revealed acute and chronic granulomatous inflammation with numerous macrophages containing many acid-fast-positive bacteria, consistent with the diagnosis of acute necrotizing mycobacterial disease. Molecular analysis of the formalin-fixed paraffin-embedded tissue identified the presence of *M. chimaera* DNA in the spleen, the liver, and the kidney. In addition, *M. chimaera* was cultured postmortem from the patient's urine. Antimicrobial susceptibility testing (AST) showed that the strain was susceptible to clarithromycin (MIC, 4 mg/liter), according to the current NCCLS AST guidelines (4). Furthermore, the MICs of ethambutol and rifabutin were 5 mg/liter and 2 mg/liter, respectively (Table 1). However, no clinical breakpoints for ethambutol, rifampin, and slow-growing nontuberculous mycobacteria currently exist.

MATERIALS AND METHODS

Organisms. The assignment of *M. chimaera* was based on 16S rRNA gene sequencing (3). The *M. chimaera* isolates investigated in this study originated from the two patients with disseminated *M. chimaera* infection as described above. In addition, eight respiratory *M. chimaera* isolates from our clinical strain collection were investigated for epidemiological purposes.

Culture techniques and antimicrobial agents. Mycobacteria were cultured by standard methods on Middlebrook 7H11 agar plates. The MICs were determined using the MGIT 960 platform equipped with the EpiCenter TB eXiST software, as described previously, using the following concentrations: 1 and 20 mg/liter rifampin, 0.1 and 2 mg/liter rifabutin, 1, 4, and 20 mg/liter amikacin, 2 and 10 mg/liter ofloxacin, 0.5, 2.5, and 10 mg/liter moxifloxacin, 4, 16, 32, and 64 mg/liter clarithromycin, 1, 2, 5, and 50 mg/liter ethambutol, 4, 8, 16, and 32 mg/liter vancomycin, and 4/4, 8/4, 16/4, and 32/4 mg/liter piperacillin-tazobactam (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) (5).

Definitions. The two *M. chimaera* blood culture isolates were considered clinically significant in light of the two case presentations. The clinical relevance of *M. chimaera* strains isolated from respiratory specimens was assessed according to the 2007 American Thoracic Society (ATS) criteria for nontuberculous mycobacterial (NTB) lung disease (6). The variables evaluated were pulmonary symptoms, nodular or cavitary opacities on a chest radiograph, multifocal bronchiectasis with small multiple nodules shown on a high-resolution computed tomography scan, positive culture results from at least two separate expectorated sputum samples, and positive culture results from at least one bronchial wash or lavage. Clinical data were retrieved from the hospital records.

Identification of *Mycobacterium* spp. and strain comparison. 16S rRNA gene sequencing from cultures was performed using primers 283 and 264 for PCR amplification and primer Mbakt-14 for sequencing (7). Sequences were analyzed using the SmartGene IDNS software and databases (SmartGene, Zug, Switzerland). RAPD-PCR for analysis of strain relatedness was performed using chromosomal DNA and the primers IS986-FP and OPA18 as described previously for *M. abscessus* (8).

Infection control provisions. Samples were taken from five different hospital sources: faucet water of the operating room, faucet water of the cardiac surgery intensive care unit, condensing water from Heather controller units (HCUs), cardioplegia solutions, and hemofiltration solutions.

RESULTS

Antimicrobial susceptibility testing. Table 1 summarizes the results of antimicrobial susceptibility testing (AST) of the two patients according to the current NCCLS AST guidelines (4). AST did not show resistance to clarithromycin as the mainstay drug in MAC infections, and although no clinical breakpoints for ethambutol, rifampin-rifabutin, and slow-growing nontuberculous mycobacteria currently exist, the rifampin-rifabutin and ethambutol MICs determined for the two strains were low, and both isolates can probably be considered susceptible. A second AST from the urine isolate of patient 2 was performed to detect the development of a possible resistance to clarithromycin that could explain the treatment failure after 9 months. However, the *M. chimaera* strain from the urine sample was susceptible to clarithromycin (MIC, 4 mg/liter). Furthermore, the MICs of ethambutol and rifabutin were 5 mg/liter and 2 mg/liter, respectively (Table 1).

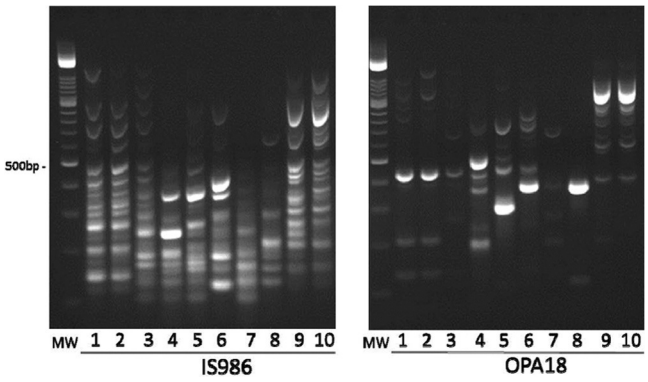

FIG 2 *Mycobacterium chimaera* strain typing using randomly amplified polymorphic DNA (RAPD)-PCR. Shown are RAPD-PCR patterns of *M. chimaera* clinical isolates from the two patients (lane 1, patient 1; lane 2, patient 2) and of eight respiratory culture isolates from eight different patients (lanes 3 to 10). RAPD-PCR patterns were generated with primers IS986-FP (A) and OPA18 (B). MW, molecular weight marker.

Epidemiological investigation. Our retrospective case study detected 8 patients with respiratory *M. chimaera* isolates. These *M. chimaera* strains were not considered clinically relevant according to the 2007 ATS criteria for NTB lung disease (6).

***M. chimaera* strain comparison using RAPD-PCR.** To date, no standard methods for the strain typing of *M. chimaera* have been described. We applied randomly amplified polymorphic DNA (RAPD)-PCR to the isolated *M. chimaera* strains with identical primers (IS986-FP and OPA18) as has been reported for *M. abscessus* (8). Gel electrophoresis of the RAPD-PCR amplicons showed identical patterns for the *M. chimaera* strains of the two case patients for both primers used (Fig. 2, lanes 1 and 2), indicating a relatedness of these strains. Eight clinical *M. chimaera* strains obtained from the respiratory tracts of eight other patients showed different patterns in comparison with the *M. chimaera* strains of the two case patients (Fig. 2). Two respiratory strains showed identical patterns (Fig. 2, lanes 9 and 10). Intensive searches performed in the hospital in 2012 did not detect an *M. chimaera* strain with an RAPD-PCR pattern identical to that of the isolates from the two case patients.

DISCUSSION

The most frequent pathogens in PVE are coagulase-negative staphylococci, *Staphylococcus aureus*, streptococci (*Streptococcus viridans* group), and *Enterococcus* spp. In up to 11% of PVE cases, blood cultures remain negative or become positive with rare infective agents of endocarditis, such as HACEK group members (*Haemophilus paraphrophilus*, *Aggregatibacter actinomycetemcomitans*, *Aggregatibacter aphrophilus*, *Cardiobacterium hominis*,

Eikenella corrodens, and *Kingella kingae*), *Propionibacterium acnes*, or mycobacteria (9). PVE due to mycobacteria most often involves environmental rapidly growing NTM, suggestive of a nosocomial waterborne source of infection. In the setting of a prosthetic valve and an infection due to waterborne pathogens, like NTM, contaminated cardioplegic and/or valve-preserving solutions and the water supply system of the operating room should be evaluated as a possible sources of infection (1). Alternatively, contamination of the sternotomy wound or colonization of the prosthetic material due to intravenous catheter-related bacteremia with NTM might be considered (10). Other authors have hypothesized that an NTM-contaminated implanted bioprosthetic valve might be the source of a mycobacterial prosthetic valve endocarditis (10, 11). The detection of NTM in cultures of valve tissue might also be false positive, e.g., represent cross-contamination with MAC-positive sputum samples processed at the same time in the microbiology lab (12). Contaminated tap water has been described as another possible source of sample contamination (2).

In the first patient described, cross-contamination was unlikely because of the concurrent detection of *M. chimaera* in the heart valve tissue, the three consecutive blood cultures, and a tracheal secretion processed in two independent microbiology laboratories. Preoperative contamination of the annuloplasty material is an unlikely source of infection because of the elaborate decontamination processes against mycobacteria performed by manufacturers. The two patients in this study received annuloplasty material from two different companies. Thus, the initial source of the NTM infection in the first patient remains unknown. Nevertheless, the initial diagnosis of systemic sarcoidosis has to be scrutinized in retrospect. Post hoc analyses of the stored FFPE samples of the previous liver and kidney biopsy specimens with granulomatous inflammation did not detect *M. chimaera* DNA. The steroid treatment might have accelerated the valve destruction and contributed to the *M. chimaera* dissemination. NTM infections are especially common in immunocompromised patients and persons with specific mutations in the gamma interferon synthesis pathway (10), a pathway that might be directly impaired by glucocorticoid administration. There was no clinical evidence of infective endocarditis in the second patient. Interestingly, the patient had an indwelling mechanical aortal prosthesis and a composite graft. This raised the issue of an epidemiological relatedness between the two patients, which we did not expect initially because of the 2-year interval between the surgeries on these two cases.

The two fatal cases with an involvement of PVE and bloodstream infection due to *M. chimaera* showing close relatedness as indicated by RAPD-PCR (Fig. 2) prompted investigations by the hospital infection control unit. Special emphasis was put on identifying a possible nosocomial source. Intensive searches in the hospital did not detect an *M. chimaera* strain with a RAPD-PCR pattern identical to that of the two patients with PVE and bloodstream infection. Although RAPD-PCR had not been used previously for comparison of *M. chimaera* strains, our results indicate that the primers used for the comparison of *Mycobacterium abscessus* strains can also be applied to other *Mycobacterium* spp., which gave the first indication of strain relatedness in the case of the identical amplicon patterns. However, we did not find similar *M. chimaera* strains in the hospital, and we cannot exclude a nosocomial infection.

M. chimaera is known to cause pulmonary disease, but until now, *M. chimaera* had not been reported as a cause of bloodstream infection or endocarditis. *M. chimaera* is a MAC sequevar with a close relationship to *M. intracellulare* (3). Due to this close relationship, *M. chimaera* strains can be falsely assigned as *M. intracellulare* (13). A study that reanalyzed 107 MAC-positive blood culture samples did not detect such *M. chimaera* strains (14). We did not find reports on PVE related to *M. intracellulare*.

The clinical utility of antibiotic drug susceptibility testing in the management of patients with NTM is controversial (6). The recommended treatment regimen for a disseminated MAC infection is the combination of clarithromycin, rifampin, and ethambutol supplemented with an aminoglycoside, depending on the clinical presentation (6). The optimal antimicrobial regimen for *M. chimaera* infections is unknown and requires further clinical outcome studies.

In conclusion, PVE and bloodstream infections with *M. chimaera* should be considered in the differential diagnosis of patients with biomechanical or mechanical prostheses who present with diagnostic criteria compatible with endocarditis and/or septicemia. The growing number of elderly and immunocompromised persons undergoing heart valve surgery might increase the probability of PVE with slow-growing NTM. A thorough histopathological examination of valve tissue and blood cultures for mycobacteria can augment the diagnosis of NTM infection.

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We declare no conflicts of interest.

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