

Mycoplasma Endocarditis: Two Case Reports and a Review

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We describe 2 patients with endocarditis for whom blood cultures and cardiac valve cultures were repeatedly sterile. Broad-range eubacterial polymerase chain reaction (PCR) amplification performed on cardiac valve specimens from these 2 patients detected DNA of *Mycoplasma hominis*, for one patient, and of *Ureaplasma parvum*, for the other patient. Three other cases of infective endocarditis caused by mycoplasmas were identified in the literature. It is important to rule out a diagnosis of mycoplasma endocarditis because the evolution of the disease may be fatal and it requires an adequate and specific antibiotic therapy.

Mycoplasma hominis and *Ureaplasma urealyticum* are inhabitants of the human genital tract [1–4]. The organism currently known as *U. ureaplasma* has been divided into 2 species: *Ureaplasma parvum* (previously *U. urealyticum* biovar 1) and *U. urealyticum* (previously *U. urealyticum* biovar 2) [5]. These mycoplasmas have been described in urogenital tract diseases, in infections in newborns, and in postoperative wound infections [2, 6]. Cardiovascular infections caused by mycoplasmas have rarely been described [3]. To date, only 3 cases of mycoplasma endocarditis have been reported in the literature [7–9]. However, this pathology has been suggested since 1978 [10]. The diagnosis is difficult to make, as these bacteria are not seen on Gram-stained smears and are not easy to cultivate. On blood agar, *Ureaplasma* species do not grow and *M. hominis* grows poorly [11]. *Mycoplasma hominis* can be recovered from automated blood cultures if sodium polyanethol sulfonate anti-

coagulant is omitted. Several studies have suggested that broad-range PCR analysis targeting the 16S rDNA sequence in cardiac valve tissue specimens is a promising tool for etiologic diagnosis of endocarditis [12–14]. By reviewing the literature and reporting 2 new cases of endocarditis caused by *Mycoplasma* species diagnosed using PCR, we would like to alert physicians to consider mycoplasmas as agents of endocarditis.

Patients and methods. Patient 1, a 33-year-old man, was hospitalized in July 2001 in Timone Hospital (Marseille, France) for dyspnea. His history included mitral valve surgery 6 months prior to hospitalization. In February 2001, the patient underwent an annuloplasty procedure for mitral valve prolapse. Several days after the surgery, he developed a fever of 38.4°C, which resolved with the prescription of nonsteroidal anti-inflammatory drugs. At that time, 2 different urine samples revealed aseptic leukocyturia. In July 2001, examination showed signs of heart failure. The patient's WBC count was $9 \text{ cells} \times 10^9/\text{L}$ and his C-reactive protein level was 40 mg/L. Findings of a transesophageal echocardiogram showed a mitral endocarditis with a dehiscence of the mitral plasty. After blood cultures were conducted, a treatment with amoxicillin and gentamycin was started. Fifteen days after the initiation of treatment, the patient underwent mitral valve replacement with a mechanical prosthesis. Blood cultures were sterile. The results of serologic examinations for Q fever and *Bartonella* were negative. Gram-staining of a smear of the valve specimen showed polymorphonuclear cells but no bacteria. The histopathological examination of the valve showed inflammatory lesions composed of fibrin and lymphocytes. Cardiac valve cultures were sterile. PCR analysis of a cardiac valve specimen, using primers targeting the 16S rDNA sequence, was positive for *M. hominis* DNA. PCR with a second set of primers targeting a different gene confirmed the first result. The patient then received doxycycline for 4 weeks and was cured. He remained healthy 2 years after therapy.

Patient 2, a 57-year-old woman, was hospitalized in January 2001 in a cardiologic hospital in Lyon, France, for dyspnea. Her history included the presence, for 13 years, of a pacemaker and the presence of an aortic valve prosthesis, with a first surgical intervention in January 1979, followed by new surgical interventions in June 1982 and January 1984, for prosthesis dehiscence. At admission, examination revealed signs of heart failure. The patient's WBC count was $5.84 \text{ cells} \times 10^9/\text{L}$ and her C-reactive protein level was 15 mg/L. Findings of a trans-thoracic echocardiogram showed aortic endocarditis and revealed that the prosthesis had been dislodged. After blood cul-

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tures were performed, the patient underwent aortic valve replacement with a mechanical prosthesis. Treatment was started using gentamycin and vancomycin. Blood cultures were sterile. The results of serologic examinations for Q fever and *Bartonella* were negative. Gram-staining of a smear sample obtained from the cardiac valve showed polymorphonuclear cells but no bacteria. The histopathological examination of the valve showed inflammatory lesions composed of lymphocytes and macrophages. Cardiac valve cultures were sterile. PCR analysis of a cardiac valve specimen, using primers targeting the 16S rDNA sequence, was performed in Lyon and identified *U. parvum*, but this result was not considered significant. The therapy was not adjusted to treat this infection. Two weeks after initiation of antibiotic therapy, the patient presented with a pneumopathy, and ceftriaxone was added to the previous therapy. No improvement was observed. Two months after the surgery, the patient experienced cardiac failure and died. Retrospectively, PCR analysis targeting 16S rDNA and another PCR analysis targeting a different gene were performed in Marseille.

DNA extracts prepared from cardiac valve specimens were amplified using PCR targeting the 16S rRNA sequence [15]. Two second sets of primers were used: for patient 1, MH1F (5'-TGAGCATATAAAAGAATTGC-3') and MH1R (5'-GTGT-TGTATCGACAA CAG-3') targeting the *M. hominis* *ftsY* gene, and for patient 2, U4 (5'-ACGACGTCCATAAGCAACT-3') and U5 (5'-CAATCTGCTCGTGAAGTATTAC-3') targeting the urease gene of *Ureaplasma* species [16]. Each experiment included a negative control group. DNA sequencing reactions were performed as described elsewhere [15].

For serologic testing, a control group of 10 serum samples—5 obtained from blood donors and 5 from patients with suspected rickettsioses—were tested in parallel with samples obtained from our 2 patients. *Mycoplasma hominis* strain PG 21 (ATCC 23114) and *U. urealyticum* strain 2K 160 (ATCC 27618) were cultivated using Urea-Arginine LYO 2 broth (bioMérieux). The antigen solution was prepared by mixing 2 parts of *Mycoplasma* antigen, adjusted to a protein concentration of 1 mg/mL, with 1 part of PBS and 1 part of the Laemmli solubilizer at room temperature [17]. Ten micrograms of protein per spot were loaded on a nitrocellulose strip (2 inches by 2 inches). The strips were then incubated with 5% (wt/vol) nonfat dry milk in Tris buffer overnight, then with 2-fold diluted serum in 0.5% (wt/vol) nonfat dry milk in Tris buffer overnight, then with 2-fold diluted serum in 0.5% (wt/vol) nonfat dry milk in PBS for 1 h at room temperature. After three 10-min washing steps in Tris buffer, the membranes were incubated with goat anti-human IgG and IgM (Jackson ImmunoResearch Laboratories) diluted to 1:200 in PBS in 0.5% (wt/vol) nonfat dry milk. After 3 washes in PBS, the bound peroxidase activity was revealed by using 4-chloro-1-naphthol as the substrate. Anti-*M. hominis* and anti-*U. urealyticum* mice polyclonal antibodies,

produced using the 2 ATCC reference strains as described elsewhere [18], were used as a positive control.

A search of the MEDLINE database for reports published during the period 1966–2001 was performed. Reports published prior to 1966 were searched by cross-referencing.

Results. For patient 1, DNA amplification of 2 different samples from the cardiac valve was performed using the 2 sets of primers. The sequences were analyzed using the Genbank database (Bethesda, MD). This resulted in the identification of an *M. hominis* isolate with 100% similarity to the respective gene sequence of *M. hominis* strain P2 (Genbank accession numbers AJ00268 and AJ298012). For patient 2, DNA was amplified from 2 different samples of the cardiac valve using the 2 sets of primers. The sequences demonstrated 99% similarity to the 16S rRNA gene and 100% similarity to the urease gene of a strain of *U. parvum*, for which the full genome has been sequenced (GenBank accession number NC-002162). All negative controls failed to yield PCR products. Moreover, within 3 years, DNA extracted from various samples has been tested 1270 times in our laboratory using 16S rDNA PCR, and none of these samples have tested positive for DNA of *M. hominis* or *Ureaplasma* species.

The results of serologic tests performed with anti-*M. hominis* and anti-*U. urealyticum* mice polyclonal antibodies were both positive, with a titer of 1:3200. Serologic testing for patient 1 was positive for *M. hominis*, with a titer of 1:1600. All the serum samples obtained from the blood donors and from the patients with suspected rickettsioses (i.e., the control group) had negative test results. The results of tests performed on serum samples obtained from patient 2 and from the control group were negative for *U. urealyticum*.

The literature review found 3 additional cases of mycoplasma endocarditis [7–9]. Pertinent clinical data from the 3 previously reported cases of endocarditis due to *Mycoplasma* species and from the 2 present cases are provided in table 1. All of these patients presented with underlying valvular abnormalities. The 2 patients whose antibiotic therapy was not adjusted to treat endocarditis due to *Mycoplasma* species died, whereas all the patients with an adjusted antibiotic therapy recovered.

Discussion. Our data confirm the existence of mycoplasma endocarditis. The diagnosis was based on the results of PCR analysis, a technique that is limited because of the risk that samples will be contaminated and results misinterpreted. In the 2 cases we report, however, this problem can be excluded. First, we had never amplified mycoplasma DNA in our laboratory before, and all of the tests performed on samples from our negative control group had negative results. Second, DNA amplification was performed using primers targeting 2 different genes. In addition, the results of serologic testing of samples obtained from one of our patients were positive, and the patient whose therapy was not adjusted to treat mycoplasma endo-

Table 1. Selected demographic data, clinical findings, and outcomes for 5 patients with endocarditis due to mycoplasmas.

Patient	Age in years, sex	Underlying condition(s)	Valve(s)	Symptom(s)	Diagnostic tool	Mycoplasmas identified	Surgery	Antibiotic therapy (duration, weeks)	Outcome	Reference
1	33, M	Mitral valve plasty	Mitral	Heart failure	PCR from cardiac valve	<i>Mycoplasma hominis</i>	Yes	Amo and Genta (3); Doxy (4)	Survival	PR
2	56, F	Prosthetic aortic valve	Aortic	Heart failure	PCR from cardiac valve	<i>Ureaplasma urealyticum</i>	Yes	Vanco (8); Genta (2)	Death	PR
3	21, M	Postrheumatic heart disease; dental extraction without antibiotic prophylaxis 4 weeks before hospitalization	Mitral	Fever, anorexia, sore throat, malaise	Serology	<i>Mycoplasma pneumoniae</i>	No	Benz and Genta (6); Oxy (4)	Survival	[7]
4	25, F	SLE treated with corticosteroids <i>Streptococcus sanguis</i> endocarditis; prosthetic aortic and mitral valves	Aortic and mitral	Fever	Culture from a portion of valve	<i>Mycoplasma hominis</i>	Yes	Clin and Rif (6); Doxy (4)	Survival	[8]
5	46, M	Prosthetic mitral valve	Mitral	Heart failure, fever	Culture from a portion of valve	<i>Mycoplasma hominis</i>	Yes	Vanco and Amik (1)	Death	[9]

NOTE. Amik, amikacin; Amo, amoxicillin; Benz, benzylpenicillin; Clin, clindamycin; Doxy, doxycycline; Genta, gentamycin; Oxy, oxytetracycline; PR, present report; Rif, rifampin; SLE, systemic lupus erythematosus; Vanco, vancomycin.

In cases of mycoplasma endocarditis, the source of the bacterial infection is difficult to know. A possible origin of bacteremia could be the colonization of the oropharyngeal, the respiratory, or the genitourinary tracts after a procedure such as intubation or insertion of a urinary catheter. Of note, patient 1 presented with aseptic leukocyturia several days after surgery, but no specific studies were performed. In addition, all of the patients with *M. hominis* endocarditis developed infections immediately following cardiac surgery, suggesting a nosocomial source of infection. Recognition of mycoplasma endocarditis is important because β -lactam and aminoglycoside antibiotics, which are usually used in the treatment of endocarditis [9], are not effective against mycoplasmas [19]. When confronted with the intrinsic resistance of *M. hominis* to erythromycin, therapy with doxycycline is the treatment of choice.

Acknowledgment

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