

Bivalvular *Bartonella henselae* Prosthetic Valve Endocarditis[∇]

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Prosthetic valve endocarditis is an uncommon manifestation of infection with *Bartonella* species. Herein, we report a case of *Bartonella henselae* endocarditis involving prosthetic mitral and aortic valves. The patient had a favorable outcome with combined medical and surgical therapy. Concomitant crescentic glomerulonephritis led to an initial mistaken diagnosis of Wegener's granulomatosis.

CASE REPORT

A 43-year-old Caucasian male with underlying mitral valve prolapse was diagnosed with culture-negative endocarditis involving his mitral and aortic valves in 1999. He required the placement of an aortic homograft and porcine mitral valve; 6 weeks of parenteral antimicrobial therapy was subsequently administered (details unknown). An etiology for his endocarditis was apparently never established by either blood or excised-valve cultures. He remained in good health after surgery.

In August 2005, the patient presented with increasing fatigue and intermittent fevers. Blood cultures were negative, and a transthoracic echocardiogram showed no abnormalities. A transesophageal echocardiogram (TEE) done for persistent fevers (October 2005) showed no evidence of endocarditis. He subsequently developed gross hematuria; cystoscopy results were within normal limits.

In November 2005, he again presented with worsening fatigue, ongoing fevers, and acute renal insufficiency. A TEE revealed a 1.5-by-0.5-cm vegetation on the porcine mitral valve and thickening of the aortic valve homograft. The results of a renal biopsy were consistent with focal segmental crescentic glomerulonephritis (GN). In addition, laboratory studies revealed proteinase 3-specific antineutrophil cytoplasmic antibodies (PR3-ANCA). Therapy with prednisone and cyclophosphamide was initiated for a presumptive diagnosis of Wegener's granulomatosis. Antimicrobial therapy with ceftriaxone, doxycycline, and daptomycin was also initiated for culture-negative endocarditis. He was transferred to a tertiary-care medical center, where multiple sets of blood cultures did not reveal any growth. The following laboratory tests were negative: serology tests for *Legionella* spp., *Coccidioides immitis*, Q fever, Whipple's disease, *Mycoplasma pneumoniae*, and human immunodeficiency virus; a *Cryptococcus neoformans* antigen test; and a PCR test for *Brucella* spp. *Chlamydia* and *Chlamydophila* spp. serology tests were positive (concentrations of immunoglobulin G [IgG] for *Chlamydophila pneumoniae*, *Chlamydia trachomatis*, and *Chlamydophila psittaci* in

serum, >1:256). *Bartonella* spp. serology analyses were not performed. The patient was treated with ceftriaxone, doxycycline, and rifampin for 6 weeks. Prednisone and cyclophosphamide were discontinued when immunofluorescence studies revealed a pauci-immune pattern not consistent with Wegener's granulomatosis.

Fevers recurred immediately after the completion of antimicrobial therapy (end of December 2005). The patient was transferred to our institution for further evaluation in February 2006. Upon admission, a cardiovascular exam disclosed an atrial flutter with a regular heart rate, a grade 3/6 early-diastolic murmur along the left sternal border, and a middiastolic murmur at the apex. TEE revealed thickened leaflets and vegetations on the aortic homograft, severe aortic regurgitation, and substantial thickening of the bioprosthetic mitral valve with mitral stenosis and vegetations. Left ventricular function was preserved.

Antimicrobial therapy was withheld, and the patient remained afebrile for the next 6 days. Nine sets of blood cultures remained negative. Results of serologic studies for potential pathogens causing culture-negative endocarditis were as follows: *Coccidioides immitis* IgG and IgM by enzyme immunoassay and complement fixation, negative; *C. pneumoniae* IgG, 1:512; *C. psittaci* and *C. trachomatis* IgG, negative; *Brucella* spp. IgG antibody, positive; *Brucella* spp. IgM antibody, negative; Q fever phase I IgG, 1:64, and phase II IgG, 1:128; Q fever phase I and II IgM, less than 1:16; *Legionella pneumophila* antibody (as tested by enzyme immunoassay), positive; *Brucella abortus* IgG and IgM (as determined by indirect fluorescent-antibody assay [IFA]), less than 1:20; and human immunodeficiency virus antibody, negative. Testing for PR3-ANCA gave positive results. The level of *Bartonella henselae* IgG was 1:1,024, and that of *Bartonella quintana* IgG was 1:16,384; levels of corresponding IgM antibodies were <1:20. Upon further questioning, the patient admitted to acquiring a kitten 6 months prior to the onset of his symptoms and had sustained scratches and bites from his kitten on a regular basis. He did not recall observing fleas on his kitten.

The patient was taken to the operating room 6 days following admission. The aortic homograft leaflets were found to be destroyed, and the bioprosthetic mitral valve had multiple vegetations, causing obstruction of the valve orifice. There was no evidence of intracardiac abscess. The diseased aortic and mi-

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tral prosthetic valves were replaced with St. Jude mechanical valves. Histopathology of the patient's mitral and aortic valves revealed gram-variable organisms upon staining of both excised mitral and aortic valves with the Gram and Warthin-Starry stains. Fungal (silver) and mycobacterial (auramine-rhodamine) staining of both valves gave negative results. Intraoperative cultures from his mitral and aortic valves (including routine, anaerobic, fungal, and mycobacterial cultures) showed no growth. Both blood and intraoperative valve cultures remained negative despite prolonged incubation.

For PCR testing, material from mitral and aortic valve specimens was digested by proteinase K digestion, and DNA was extracted using the MagNA Pure system (Roche Applied Science). *Escherichia coli* (ATCC 25922) in S.T.A.R. buffer (Roche Applied Science) was used as a negative extraction control, and a plasmid template clone of the 250-bp template in S.T.A.R. buffer was used as the positive control.

The reaction mixture for testing on a LightCycler instrument was prepared in a controlled-access reagent preparation room and consisted of 15 μ l of the PCR master mix plus 5 μ l of the DNA extracts from the valve tissue specimens per cuvette. A portion of the citrate synthase gene (*gltA*) of *B. henselae* was used as a target sequence. The sequence of the forward primer, bart 430, was 5' GCA TTC TAT CAC GAC TC 3', and the sequence of the reverse primer, bart 680, was 5' TTG TTC ATG ATC TGC AT 3'. The sequence of the anchor probe (fluorescein labeled), bartCS-F, was 5' CAA GCA TTT GTT TAT CCA CGT AAT 3', and the donor probe sequence (labeled with LC Red 640), bartCS-RM, was 5' TCT TAG TTA CGC TGC AAA TTA TCT CC 3'. A base substitution in the donor probe sequence was employed to achieve greater separation in the melting-curve analysis between *B. henselae* and *B. quintana*. The target sequence generated by the primer and probe sequences was checked for homology to unrelated sequences by a BLAST search of the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>). Version 3.5 of the LightCycler software was used for data analysis.

As a means of verification, direct 16S rRNA gene sequencing of the mitral and aortic valve specimens was also performed as described elsewhere (19). Tissue extracts were tested using the MicroSeq microbial identification system (Applied Biosystems). Further analysis of the consensus sequence was performed by comparing sequences within the NCBI database by using BLAST. *B. henselae* and *B. quintana* sequences had <1% total error within the top 10 NCBI BLAST matches.

The LightCycler assay melting-point criteria confirmed biventricular endocarditis with *B. henselae*. PCR amplification and 16S rRNA gene sequencing of the valve tissues also positively identified *Bartonella* infection.

Postoperatively, the patient was treated using a regimen of gentamicin, doxycycline, vancomycin, and ceftriaxone. Gentamicin was administered for 2 weeks. He remained on doxycycline for 1 year after his surgery. He was advised to avoid contact with cats. The patient is doing well 18 months after valve surgery.

Positive blood cultures are one of the major criteria for the diagnosis of infective endocarditis (IE). The incidence of neg-

ative blood cultures in patients with endocarditis ranges from 2.5 to 48% (5). With the advent of novel and improved culture techniques, serology, and molecular methods for the identification of organisms, the true incidence of culture-negative IE is diminishing. In a recent study of 348 patients in France with culture-negative IE, patients underwent an extensive battery of tests, including serologic studies, shell vial cultures, and analyses of valve specimens by culture, microscopy, and PCR amplification. An etiology was established in 275 (79%) of the cases; *Coxiella burnetii* (48%) and *Bartonella* species (28%) accounted for the majority. Finally, only five cases (1%) were truly unexplained (10).

Bartonella spp. are small, fastidious, gram-negative, intracellular bacteria and constitute a common cause of true culture-negative IE. To date, six species, *B. quintana*, *B. henselae*, *B. elizabethae*, *B. vinsonii* subsp. *berkhoffii*, *B. koehlerae*, and *B. alsatia*, have been known to cause human IE (1, 4, 18). *B. quintana* (the causative agent of trench fever) accounts for 75% of the cases, while *B. henselae* (the causative agent of cat scratch disease) accounts for the remaining 25%; the other species are rarely implicated (10, 16). *B. quintana* IE is associated with homelessness, poor living conditions, chronic alcoholism, and exposure to body lice, while *B. henselae* IE occurs following exposure to cats or cat fleas (7, 10). Up to 40% of domestic cats in the United States have been shown to harbor *B. henselae*. Underlying valvulopathy is significantly more common with *B. henselae* IE than *B. quintana* IE (10).

There are several methods available to establish a diagnosis of *Bartonella* IE: tissue cell culture (with the shell vial technique), Warthin-Starry staining of the resected valve, immunohistologic methods, serologic testing, and PCR amplification using valve specimens have been used alone or in combination (4, 5). Specific antibodies against *Bartonella* spp. can be detected by IFA and enzyme-linked immunosorbent assay. An IFA titer of >1:800 against *Bartonella* spp. has been shown to have a 95% predictive value for *Bartonella* IE. Hence, its inclusion as a major Duke's criteria has been suggested (8). It is worth noting that low-level cross-reactivity with *Chlamydia* spp. and *Coxiella burnetii* (as seen in the present case) in patients with *Bartonella* infection has been described previously (5, 13). IFA cannot reliably distinguish between antibody responses to *B. henselae* and *B. quintana* (5); this was demonstrated in our case, in which titers of antibodies to *B. quintana* were higher than *B. henselae* antibody titers despite an established diagnosis of *B. henselae* infection. Antibody cross-absorption and Western immunoblotting can allow differentiation of serological responses to *B. henselae* and *B. quintana* (5); these steps were not performed in our case.

Molecular diagnostic techniques utilizing PCR amplification and direct sequencing of DNA from resected valve specimens can achieve a specificity approaching 100% in the diagnosis of culture-negative IE (5). The use of broad-spectrum primers can detect almost all bacteria in a single reaction. Specific primers against most bacterial genera, including several pathogens implicated in culture-negative IE (4), are also available. The LightCycler technology for the diagnosis of *Bartonella* infection was developed and extensively validated at Mayo Clinic (Rochester, MN) prior to clinical use. It combines the features of rapid PCR and real-time detection of an amplified target. By using a portion of the citrate synthase gene (*gltA*),

TABLE 1. Reported cases of *Bartonella* prosthetic valve endocarditis

Reference	Age of patient (yrs)	Sex ^a	<i>Bartonella</i> species	Valve(s) involved (type)	Valve surgery	Outcome
Klein et al. (11)	46	F	<i>B. quintana</i>	Aortic (mechanical)	Yes; replacement with aortic homograft	Alive 6 wks after surgery
Lesprit et al. (14)	70	F	<i>B. henselae</i>	Aortic (bioprosthetic)	No	Alive 40 mos after diagnosis
Kreisel et al. (12)	36	M	<i>B. henselae</i>	Mitral (porcine)	Yes; replacement with mechanical mitral valve	Alive
	76	M	<i>B. henselae</i> or <i>B. quintana</i> (?)	Mitral (porcine)	No	Died
Sondermeijer et al. (20)	45	M	<i>B. quintana</i>	Aortic and mitral (mechanical)	No	Died
Walls et al. (21)	10	F	<i>B. henselae</i>	Pulmonary (homograft)	Yes; replacement with pulmonary homograft	Alive 8 mos after surgery
Present study	43	M	<i>B. henselae</i>	Aortic (homograft) and mitral (porcine)	Yes; replacement with mechanical aortic and mitral valves	Alive 18 mos after surgery

^a F, female; M, male.

the causative species of *Bartonella* can be accurately identified. Both of these molecular techniques can provide useful etiologic information in cases in which blood and valve cultures remain negative.

The optimal antimicrobial therapy for *Bartonella* IE remains unclear. Against *B. henselae*, macrolides, tetracycline, and rifampin are particularly effective, while ciprofloxacin and gentamicin have slightly higher MICs (15). Only aminoglycosides are considered bactericidal toward *Bartonella* spp., and a minimum 2-week course of aminoglycoside therapy has been associated with favorable outcomes (17). Recent guidelines for the diagnosis and management of IE recommend doxycycline for 6 weeks and gentamicin for the initial 2 weeks of therapy for documented *Bartonella* IE (2). If gentamicin cannot be administered, then rifampin should be the substitute. Valve replacement surgery may be necessary in up to 80% of cases due to extensive valvular damage (4).

The majority of reported cases of *Bartonella* endocarditis affect native valves. In the English-language literature, there are six previous reports of cases of prosthetic valve endocarditis due to *Bartonella* species (Table 1). Patients in three of the reported cases, in addition to the patient in the present case, underwent valve replacement surgery with favorable outcomes.

A review of renal biopsy findings in *B. henselae* native-valve endocarditis with associated GN revealed a pattern of immune complex-mediated GN with segmental necrotizing and crescentic lesions (3). It is unclear if *Bartonella* directly infects the kidneys, with resulting rapidly progressive renal failure, since renal biopsy specimens were not examined for *Bartonella* in any of the reported cases (including ours). PR3-ANCA-positive GN and vasculitis-like illness in patients with underlying endocarditis have been well described previously (6, 9).

Our case demonstrates several unique aspects of *B. henselae* endocarditis. (i) To our knowledge, this is the first case of established *B. henselae* endocarditis involving prosthetic aortic and mitral valves. (ii) This is also the only case in which the patient is alive and well more than 18 months after both the diseased prosthetic valves were replaced and prolonged anti-

microbial therapy was administered. (iii) The IFA-measured titers of antibodies to *B. quintana* were severalfold higher than those of *B. henselae* antibodies despite an established diagnosis of *B. henselae* endocarditis, due to the inability of IFA to distinguish between *B. henselae* and *B. quintana* antibodies. (iv) The diagnosis of *B. henselae* endocarditis was established by rapid real-time PCR using LightCycler technology and verified by direct 16S rRNA gene sequencing of the valve specimens. (v) Positive PR3-ANCA results, renal failure, and crescentic GN led to an initial mistaken diagnosis of Wegener's granulomatosis. This case highlights the importance of considering endocarditis in the differential diagnosis of PR3-ANCA-positive crescentic GN despite negative blood cultures; immunosuppressive therapy in the absence of antibiotics may have resulted in detrimental outcomes.

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