Tropheryma whippelii Infective Endocarditis as the Only Manifestation of Whipple's Disease[▽]

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Here we describe a case of infective endocarditis caused by *Tropheryma whippelii* in a patient with no other symptoms of Whipple's disease. The case was diagnosed using broad-range PCR and confirmed by specific PCRs. We review the cases of infective endocarditis presenting as the only manifestation of Whipple's disease reported in the literature.

CASE REPORT

A 51-year-old man was admitted to a hospital with a 1-month history of low-grade fever, dyspnea, and ankle edema. His medical history was unremarkable, with no previous heart valve damage. Physical examination revealed a temperature of 37.2°C and grade II/VI systolic and diastolic murmurs. Laboratory analysis revealed a high white blood count with left-side deviation. Electrocardiography showed that the patient was in sinus rhythm at 95 beats per minute. A transesophageal echocardiogram revealed two vegetations, one on the aortic valve, measuring 22 mm at the maximum point, and a smaller one on the anterior leaflet of the mitral valve. Three sets of aerobic and anaerobic blood cultures (plus BACTEC aerobic/F and anaerobic/F bottles) drawn before antimicrobial therapy was started were negative after 20 days of incubation using a BACTEC 9240 system (Becton-Dickinson, Sparks, MD). Empirical therapy consisted of diuretics, captopril, gentamicin (100 mg/8 h), and vancomycin (1,000 mg/12 h).

The patient underwent heart surgery (aortic valve replacement with a prosthetic valve and mitral repair) due to heart failure 5 days after a confirmation of the diagnosis of infective endocarditis (IE) by echocardiography. Intravenous ceftriaxone (2 g/day) was added to previous antimicrobial therapy to cover the agents responsible for culture-negative IE.

Macroscopically, the resected valve showed no abscesses or communications, but two vegetations were observed. Gram stain and a conventional microbiological culture of valve tissue were negative after 20 days of culture in sheep blood agar, brain heart broth, chocolate agar in a 5% CO₂ atmosphere, and *Brucella* agar in an anaerobic atmosphere. All cultures were incubated at 37°C. Histological examination showed findings compatible with IE, but no other alterations.

To detect possible fastidious bacteria, PCR for amplification of the bacterial 16S rRNA gene in valve tissue was performed by real-time PCR in a LightCycler instrument using SYBR

green I (Roche Diagnostics) and broad-range primers PSL (forward, 5'-AGG ATT AGA TAC CCT GGT AGT CCA-3') and P13P (reverse, 5'-AGG CCC GGG AAC GTA TTC AC-3') (24). The human β -globin gene was detected in parallel in each PCR as a PCR inhibitor control (7). DNA was extracted from valve tissue with the QIAamp tissue DNA minikit (QIAGEN Ltd., United Kingdom). At the 17th cycle, the PCR produced an amplicon of 607 bp, characterized by a melting temperature of 90.02°C, which was subsequently sequenced using the same primers with the BigDye terminator method and detected in an ABI Prism 3100 automatic DNA sequencer (Applied Biosystems, Inc.).

The sequences obtained were compared with those stored in GenBank databases, using BLAST software (version 2.0; National Center for Biotechnology Information). Identification to species level was defined in accordance with previously published criteria as >99% sequence similarity with a high score (9). This search identified the bacterium as *Tropheryma whippelii*, with 100% similarity to sequences of *T. whippelii* AE 016850.1 and X99636.2 deposited in the GenBank and EMBL databases, respectively.

PCR using primers W3FE (5'-GGAATTCCAGAGATACG CCCCCGCAA-3') and W2RB (5'-CGGGATCCCATTCGC TCCACCTTGCGA-3'), specific for the *T. whippelii* 16S rRNA gene, was performed to verify this result (21). A seminested PCR to detect the *T. whippelii* *hsp65* gene with primers whipp-frw1 (5'-TGACGGGACCACAACATCTG-3'), whipp-frw2 (5'-CGCGAAAGAGGTTGAGACTG-3'), and whipp-rev (5'-ACATCTTCAGCAATGATAAGAAGTT-3') was also performed (18). The amplicons obtained were sequenced once again to confirm the previous result. Positive PCR results due to carryover contamination were ruled out by using negative controls in all experiments and good laboratory practices and because no previous positive amplification for *T. whippelii* had been obtained in our laboratory.

In order to characterize the strain of *T. whippelii* detected in our patient's valve tissue, the 16S-23S rRNA gene intergenic spacer region and domain III of the 23S rRNA gene were amplified and sequenced as previously described (11, 12) and classified as type 1A.

Universal and specific PCRs were also performed on DNA

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extracted with the QIAamp blood DNA minikit (QIAGEN, Ltd., United Kingdom) from blood culture supernatants and patient EDTA whole blood taken under treatment. No amplification was produced.

After a positive PCR result for *T. whipplei* was obtained, valve tissue was reexamined in the pathology laboratory and periodic acid-Schiff stain (PAS)-positive, diastase-resistant inclusions were detected inside macrophages. Both findings are characteristic of, but not completely specific to, Whipple's disease (16).

Thoracic, abdominal, and cranial computed tomography scans were performed to investigate extracardiac involvement of Whipple's disease; because no pathological findings were observed, no further invasive procedures were considered ethical at that time.

During the follow-up, the patient had gastritis symptoms (probably related to the therapy), and an upper-tract endoscopy and a colonoscopy were performed. No histopathological or molecular data suggestive of Whipple's disease were found.

The patient completed a 12-month treatment with oral cotrimoxazole and has progressed satisfactorily.

A review of the literature was carried out for IE caused by *T. whipplei*. Using the words Whipple or *Tropheryma* as keywords, a Medline search of reports that were published from January 1966 to July 2005 in PubMed (<http://www.ncbi.nlm.nih.gov/>) provided 2,546 references. The word endocarditis was included in 48 of the references. Of these, 18 references described one or more cases of IE in patients with no evidence of intestinal involvement. No involvement other than the heart was detected in nine patients (3, 5, 8, 10, 14, 19, 22, 23), and we were able to obtain minimal data for eight, as shown in Tables 1 and 2. We have added our own case to the tables.

In the review, all patients but one were middle-aged men. Valvular involvement included the aortic valve in five patients and the mitral valve in two patients, and in the remaining two patients (including our case), both the aortic and mitral valves were involved. All were native valves except for one bioprosthetic porcine aortic valve. Clinical manifestations were associated with heart failure, and all patients required surgical treatment. Other common symptoms were weight loss (four patients) and anemia (three patients). Three patients were afebrile in the clinical examination. Vegetations present on echocardiography were described for five patients. In five patients, antibiotic treatment with cotrimoxazole was extended for 12 months. In seven patients, a good outcome after treatment was reported. No recurrences or deaths were described for any of these patients at the time of publication.

With regard to laboratory diagnosis, characteristic PAS-positive inclusions were observed in resected heart valve tissue in six cases. In two cases, rod-shaped bacilli were detected by electron microscopy, and in the remaining case, only inflammatory signs were found. Molecular diagnosis by PCR was used in all cases, universal PCR alone was used in three cases, universal PCR confirmed by specific PCR was used in four cases (including ours), specific PCR only was used in one case, and the PCR method was not specified in the remaining case.

has been estimated that in 2 to 31% of IE cases, cultures are negative despite the use of appropriate laboratory techniques (13). This could be due to the presence of fastidious microorganisms or antimicrobial therapy when specimens are obtained. The role of molecular methods in identifying new, difficult-to-culture microorganisms is becoming clearer and enables us to improve the diagnosis of culture-negative IE (2, 9).

Whipple's disease is a rare infectious disease caused by the difficult-to-culture bacteria *T. whipplei*, which usually presents with intestinal and joint disorders, weight loss, fever, lymphadenopathy, and arthritis (16). Cardiac and central nervous system manifestations have been described for Whipple's disease, but they usually present in patients along with other conditions.

A literature review revealed 18 references describing cases of IE caused by *T. whipplei* with no overt gastrointestinal disease and nine cases of IE with no other clinical signs of Whipple's disease (3, 5, 8, 10, 14, 19, 22, 23).

Whipple's IE has been reported mainly for middle-aged men and affects healthy or previously damaged native valves. There are two published cases involving bioprosthetic material (3, 20), but in only one was IE the only apparent manifestation of Whipple's disease (3).

Infective endocarditis as the only manifestation of Whipple's disease is quite rare and was principally diagnosed postmortem until the introduction of PCR-based methods (6).

The diagnosis of Whipple's disease is complicated because clinical symptoms are not specific, and *T. whipplei* is very difficult to culture. In cases of gastrointestinal disease, diagnosis is based on the visualization of PAS-positive microorganisms inside the macrophages. Other approaches involve demonstrating the presence of *T. whipplei* by electron microscopy or by the detection of its DNA, using specific PCRs on biopsied tissue. Diagnosis is much more difficult when only extraintestinal manifestations are present, since there is no easily available serological or molecular method to detect this microorganism in blood.

The routine use of universal 16S rRNA gene PCR to study heart valve tissue for culture-negative infective endocarditis could make it possible to discover unexpected microorganisms not covered by conventional empirical therapy (7, 9); however, it is important to consider that universal PCR is prone to contamination, and false-positive results must be extensively ruled out using PCR-negative controls and good operating procedures. Each positive result should be confirmed by a second target-specific PCR, and amplicons obtained should be characterized by sequencing. Similarly, negative results should be confirmed by ruling out the presence of PCR inhibitors, using PCR internal controls or β -globin gene detection. PCR inhibitors (ethanol residues in the DNA extraction procedure, hemoglobin, paraffin in fixed tissues, etc.) could lead to a false-negative result.

Our review illustrates that *T. whipplei* should not be overlooked in the differential diagnosis of culture-negative IE, even when no other symptoms of Whipple's disease are present. Our case and those in the series reviewed suggest, as proposed elsewhere (1, 15, 17), that a positive universal or specific PCR result for a microorganism in excised heart valves should be included as one of the main Duke's criteria for diagnosis of IE.

TABLE 1. Characteristics of patients with *T. whipplei* infective endocarditis and no other manifestations of Whipple's disease^a

Patient no.	Age/sex	Underlying diseases/factors	Heart valve involved	Clinical manifestations	Symptoms onset	Presence of echocardiographic vegetations	Antibiotic therapy	Outcome ^b	Reference
1	43/M	Lower-extremity deep venous thrombosis, cerebral artery infarct	Aortic/mitral	Fatigue, wt loss, dyspnea, recurrent low-grade fever, anemia, cervical lymphadenopathies, afebrile	8 mo prior	Yes	Minocycline for an undescribed duration, cotrimoxazole for an undescribed duration	Afebrile after 7 mo	5
2	42/M	Mental retardation, severe aortic insufficiency	Aortic	Worsening congestive heart failure, wt loss, nausea and vomiting	6 mo prior	Yes	ND	Well after 9 mo	19
3	78/M	ND	Mitral	Congestive heart failure and angina	5 yr prior	No	Vancomycin/gentamicin for an undescribed duration	ND	23
4	69/M	Diabetes, myocardial infarction, hypertension, brain infarct	Aortic	Weight loss, dyspnea, anemia	6 mo prior	No	Cotrimoxazole for 12 mo	Well, no anemia	8
5	65/M	Bicuspid aortic valve	Aortic	Severe aortic insufficiency, congestive heart failure, wt loss, anemia, fatigue, malaise, afebrile	6 mo prior	ND	Cotrimoxazole for 12 mo	Well	22
6	51/M	Bicuspid aortic valve, hypertension	Aortic	Brain, renal, and splenic infarcts, afebrile	ND	Yes	Vancomycin/gentamicin for an undescribed duration, cotrimoxazole for 12 mo	Well	22
7	80/F	Bioprosthetic valve	Aortic	Prosthetic valve failure, afebrile, myalgia	2 mo prior	No	Ceftriaxone for 2 wk, cotrimoxazole for 12 mo	Well	3
8	42/M	Rheumatic heart disease	Mitral	ND	ND	Yes	Vancomycin/doxycycline/ofloxacin for an undescribed duration	ND	14
9	51/M	None	Aortic/mitral	Low grade fever, dyspnea	1 mo prior	Yes	Vancomycin/gentamicin/ceftriaxone for 22 days, cotrimoxazole for 12 mo	Well	This report

^a F, female; M, male; ND, not described in the reference.^b Outcome at the time of publication.

TABLE 2. Pathological features of the heart valve and molecular diagnostic tools used for heart valve tissue from patients with *T. whipplei* infective endocarditis and no other manifestations of Whipple's disease^a

Patient	Heart valve pathological analysis	Molecular diagnostic tool(s)	Other method	Reference
1	Macrophages with PAS-positive inclusions	PCR (not specified)	ND	5
2	Macrophages with PAS-positive inclusions, immunohistology, electron microscopy showing bacilli in the macrophages	Specific PCR (<i>rpoB</i> , ITS)	Cell culture	14
3	Macrophages with PAS-positive, rod-like organisms	Universal PCR	ND	23
4	Macrophages with PAS-positive inclusions	Universal PCR	ND	8
5	Slender gram-variable bacilli, long slender microorganism with a trilamellar membrane in electron microscopy	Universal PCR, specific PCR (<i>hsp65</i>)	ND	22
6	Inflammatory signs	Universal PCR, specific PCR (<i>hsp65</i>)	ND	22
7	Electron microscopy showed rod-shaped bacilli	Universal PCR, specific PCR (ITS and <i>hsp65</i>)	ND	3
8	Foamy macrophages, PAS-positive bacilli	Universal PCR	ND	19
9	Macrophages with PAS-positive inclusions	Universal PCR, specific PCR (16S rRNA and <i>hsp65</i>)	ND	This report

^a ITS, internal transcribed spacer; ND, not described in the reference.

Nucleotide sequence accession number. The *T. whipplei* sequence obtained was deposited in GenBank with accession number DQ065849.

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