Case Report

Detection of *Cardiobacterium valvarum* in a patient with aortic valve infective endocarditis by broad-range PCR

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Cardiobacterium valvarum, a fastidious Gram-negative bacterium, was detected in the aortic valve of a previously healthy 63-year-old man by broad-range PCR and 16S rRNA gene sequencing. In contrast to the patients in five previously published cases, our patient had neither a congenital bicuspid nor a prosthetic aortic valve. Here, we present a case of *C. valvarum* native tricuspid aortic valve infective endocarditis and a review of the literature.

Introduction

Members of the genus *Cardiobacterium* (*Cardiobacterium hominis*, *Cardiobacterium valvarum*) are a common part of the normal human oropharyngeal flora, but they can rarely act as pathogens if the mucosal integrity is disturbed (Gatselis *et al.*, 2006). Since the first *C. hominis* isolation from a patient with infective endocarditis (IE) in 1962, only 87 cases have been reported in the literature (Brouqui & Raoult, 2001; Apisarnthanarak *et al.*, 2002; Lesimple *et al.*, 2002; Balcou-Leroy *et al.*, 2003; Arnold *et al.*, 2004; Walkty, 2005; Shivaprakasha *et al.*, 2007; Jenssen *et al.*, 2008). *C. valvarum* was first described in 2004, and five cases of IE caused by this species have been published so far (Han *et al.*, 2004; Hoover *et al.*, 2005; Bothelo *et al.*, 2006; Geißdörfer *et al.*, 2007; Gonzales *et al.*, 2007). All of these *C. valvarum* IE patients had some form of pre-existing cardiac disease (congenital bicuspid aortic valve in four and prosthetic aortic valve in one), while 4/5 had histories of dental manipulation or poor teeth and 3/5 were afebrile. *C. valvarum* was not specifically identified in blood from any of the patients, but *Cardiobacterium* sp. was found in 2/5, HACEK in 1/5 and a Gram-negative bacterium in 1/5. In one case, *C. valvarum* was identified incorrectly as *Eubacterium tenue*. All cultures from resected valves were negative (3/3).

Here, we report a case of *C. valvarum* IE affecting a normal (tricuspid) aortic valve in a patient with no history of recent dental procedure that was detected by broad-range PCR and 16S rRNA gene sequencing.

Case report

A 63-year-old man was admitted to the University Hospital Brno with an approximate 1-month history of gradually developing resting breathlessness and repetitious episodes of cardiac decompensation. Chest pain, palpitation and weight loss were absent. The patient's personal history included type 2 diabetes, hypertension and cataract surgery, but no previously documented cardiac failure. He was not a drug addict and had not undergone any recent dental manipulations.

On admission, his temperature was normal, his blood pressure was 96/30 mmHg and his heart rate was 90 beats min⁻¹. An auscultation revealed both systolic and diastolic aortic murmurs and rales at the lung bases. A transoesophageal echocardiogram showed a mobile element of 20 × 5 mm on the partially destroyed tricuspid aortic valve resulting in severe aortic valve regurgitation and mild functional mitral and tricuspid valve regurgitations. An angiogram showed no significant coronary stenosis, but right heart catheterization revealed severe lung hypertension. No oral abscess was found upon dental examination. Laboratory results indicated 12.4 g haemoglobin dl⁻¹, a white blood cell count of 11 100 cells mm⁻³ and a platelet count of 287 000 mm⁻³. The C-reactive protein level was 33.9 mg dl⁻¹.

Two blood samples were drawn for culturing at different times before starting intravenous treatment with a combination of ampicillin and sulbactam at 3 g every 8 h. Cultivation was performed with a BacT/ALERT set (bioMérieux). All aerobic sets showed a Gram-negative bacterium within 4 days. Subcultures were plated on sheep

Abbreviation: IE, infective endocarditis.

blood agar (Merck) and MacConkey agar (Oxoid). Colonies of Gram-negative bacteria appeared on blood agar within 48 and 72 h from the first and second blood sample, respectively. The MacConkey agar remained negative after 6 days of cultivation. Biochemical tests confirmed the presence of Gram-negative, non-fermentative, non-haemolytic rods. The strains were susceptible to cefotaxime, amoxicillin-clavulanate, cefoperazone-sulbactam, piperacillin-tazobactam, ceftiofime and meropenem. However, a final identification of the bacteria using the BBL Crystal E/NF (Becton Dickinson) and the API ID 32 GN (bioMérieux) systems failed. Anaerobic cultures remained negative for all blood samples.

Considering the transoesophageal sonography findings and the blood culture results, our patient was diagnosed as having definite IE according to the modified Duke criteria (Li *et al.*, 2000).

On hospital day 20, surgical treatment was undertaken because of grade III–IV aortic regurgitation and heart failure. The aortic valve was replaced with a mechanical prosthetic valve (Sorin Bicarbon 23), and tricuspid annuloplasty (Carpentier Edwards 34) because of the annulus dilatation was performed at the same time. The macroscopic findings observed during surgery were consistent with a diagnosis of IE. The aortic valve was severely damaged: the tissue was necrotic with two 20-mm-long attached flying vegetations. Aortic valve tissue was sampled for both culture and molecular detection of pathogenic micro-organisms.

For molecular examination, DNA was extracted using a QIAmp DNA Mini kit (Qiagen) according to the manufacturer's instructions. PCR amplification of the 16S rRNA gene was carried out using two pairs of universal primers covering the V8–V9 and V3–V4 regions, respectively. PCR mixes were decontaminated with 8-methoxypsoralen and UV light cross-linking before template DNA was added and controls for potential PCR inhibitors and contamination were performed (Grijalva *et al.*, 2003). Bands of 372 bp and 473 bp were cut out and sequenced on an ABI PRISM 3100 sequencer (Applied Biosystems). The highest 16S rRNA gene homology was shown for *C. valvarum* (99% with GenBank accession no. AF506987 and 98% with GenBank accession no. DQ645464 for the V8–V9 and V3–V4 regions, respectively), using the BLAST tool (<http://www.ncbi.nlm.nih.gov/blast>). The result was obtained within 24 h after delivery of the sample to the laboratory.

For valve culturing, the tissue was homogenized and plated on sheep blood agar (Imuna), sheep blood agar with 10% sodium chloride (Imuna), sheep blood agar with amikacin (Imuna), chocolate agar (HiMedia) and Endo agar (Imuna) for aerobic cultivation, and on VL sheep blood agar (Imuna) for anaerobic cultivation. All media were incubated at 37 °C and remained negative for 6 days.

Postoperatively, the patient was treated with 3 g intravenous cefotaxime every 6 h for a period of 4 weeks, combined with

240 mg gentamicin every 24 h for 10 days, followed by 500 mg peroral cefuroxime every 12 h for the next 3 weeks. One year after valve replacement surgery, the patient was in good shape and classified in New York Heart Association Class I. The mechanical prosthetic valve was fully functional and no neurological sequelae were observed.

Discussion

C. valvarum is a member of the HACEK group, grows best on sheep blood agar with CO₂, and visible colonies appear after 2–3 days of incubation under optimal conditions (Han *et al.*, 2004). *C. valvarum* is more fastidious than *C. hominis*. A weakly α -haemolytic strain of *C. valvarum* with variable indole production has been documented (Geißdörfer *et al.*, 2007), although these features had previously been regarded as key factors discriminating *C. valvarum* from *C. hominis* (Bothelo *et al.*, 2006; Han & Falsen, 2005; Hoover *et al.*, 2005). Thus there seems to be no reliable phenotypic or biochemical test that can differentiate between the two *Cardiobacterium* species.

A total of six *C. valvarum* cases (including our case) have been reported in the literature to date. Cultures of the explanted valve were negative in all cases. These results were probably influenced by the administration of antibiotics before and during replacement surgery. All patients were treated with β -lactam antibiotics, which are effective in *C. valvarum* infection (Gonzales *et al.*, 2007). *C. hominis* is a rare producer of β -lactamase (Gatselis *et al.*, 2006), a characteristic that has not been documented in *C. valvarum* strains.

As in the majority of the published cases, our patient had no significant medical history, and the course of endocarditis was afebrile and insidious. All of the six patients were men with a mean age of 50 years. Endocarditis was typically associated with the presence of large, friable vegetations. During the operations, a massive inflammatory destruction of the native aortic valve necessitating valvular replacement was noted in all cases. In contrast to the previous cases, in which the patients had a congenital bicuspid aortic valve or a prosthetic aortic valve as a risk factor, our patient had none of these predispositions. Three of the six cases were complicated by heart failure and neurological complications.

Five of the six (83%) patients were cured. In one case, the patient died from severe septic shock 1 day after replacement surgery (Geißdörfer *et al.*, 2007). The surroundings of the aortic valve prosthesis were affected by a massive inflammatory process and a large abscess near the annulus in this patient. Neurological examination showed ischaemic areas in the cerebellum. This patient had no significant treatment in the oral cavity or dental procedure before the onset of the disease.

A characterization of a total of six cases of IE due to *C. valvarum* and their comparison with a total of 61 cases of *C. hominis* IE (as reviewed by Malani *et al.*, 2006) is shown in Table 1. The course of the disease (including

Table 1. Comparison of the characteristics of infective endocarditis due to *C. valvarum* and *C. hominis*

Characteristics	<i>Cardiobacterium valvarum</i> [no. (%)]*	<i>Cardiobacterium hominis</i> [no. (%)]†	P‡
Mean age	49.5 years	50.2 years	—
Men/women	6/0	42/19	NS
Symptoms	n=6	n=58	
Fever	2 (33)	43 (74)	0.058
Fatigue	3 (50)	31 (53)	NS
Weight loss/anorexia	2 (33)	23 (40)	NS
Risk factor	n=6	n=61	
Cardiac disease	0	37 (61)	0.006
Prosthetic valve	1 (16)	17 (28)	NS
Bicuspid aortic valve	4 (67)	4 (7)	0.001
Prior rheumatic fever	0	12 (20)	NS
Recent dental procedure or poor dentition	4 (67)	18 (30)	NS
Prior endocarditis	0	4 (7)	NS
Site of infection	n=6	n=61	
Aortic	6 (100)	24 (39)	0.006
Mitral	0	19 (31)	NS
Mitral and aortic	0	8 (13)	NS
Pulmonic	0	1 (2)	NS
Tricuspid	0	1 (2)	NS
Not specified	0	8 (13)	NS
Physical findings	n=6	n=53	
Murmur	3 (50)	50 (94)	NS
Mitral regurgitation	0	28 (46)	0.03
Aortic regurgitation	3 (50)	21 (34)	NS
Treatment	n=6	n=58	
Surgical replacement	6 (100)	27 (47)	0.01

*Data from Han *et al.* (2004), Hoover *et al.* (2005), Bothelo *et al.* (2006), Geißdörfer *et al.* (2007), Gonzales *et al.* (2007) and our case.

†Malani *et al.* (2006).

‡P-value was calculated using Fisher's exact test. Values ≥ 0.06 are designated not significant (NS).

predisposing factors) was very similar with both of these related pathogens. Although a very limited number of *C. valvarum* IE cases have been reported in the literature so far, and results must be interpreted very carefully, some differences seem to be apparent. These include a higher likelihood of an afebrile course, the absence of pre-existing cardiac disease, and exclusive involvement of the aortic valve (which was more frequently bicuspid) in *C. valvarum* compared to *C. hominis* IE cases. *C. valvarum* also appears to be more aggressive than *C. hominis*, as all patients with *C. valvarum* IE required surgical valve replacement, compared with only 47% of *C. hominis* IE patients requiring so (see Table 1). Taking into account the fact that discrimination of these species by conventional microbiological approaches is very difficult, a portion of the cases reported in the past as *C. hominis* IE could in fact have been caused by *C. valvarum*, particularly the afebrile cases with massive inflammatory destruction of the aortic valve requiring surgical treatment.

In conclusion, *C. valvarum* is a recently described IE-causing pathogen that is fastidious, difficult to culture and

barely distinguishable from *C. hominis*. In our case, blood samples collected for culture were positive after 4 days of incubation, but further identification of the Gram-negative rods failed. The use of 16S rRNA broad-range PCR combined with DNA sequencing allowed rapid and accurate identification of the pathogen, which is particularly helpful in cases of fastidious and/or rare pathogens causing culture-negative IE. A molecular approach was again shown to be an efficient and useful tool for pathogen detection in IE patients.

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