CASE REPORT

Culture-negative endocarditis diagnosed using 16S DNA polymerase chain reaction

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16S DNA polymerase chain reaction (PCR) is a molecular amplification technique that can be used to identify bacterial pathogens in culture-negative endocarditis. Bacterial DNA can be isolated from surgically excised valve tissue or from blood collected in EDTA vials. Use of this technique is particularly helpful in identifying the bacterial pathogen in cases of culture-negative endocarditis. A case involving a 48-year-old man who presented with severe aortic regurgitation and a four-month prodrome of low-grade fever is reported. Blood and valve tissue cultures following valve replacement were negative. A valve tissue sample was sent for investigation with 16S DNA PCR, which successfully identified *Streptococcus salivarius* and was interpreted as the true diagnosis. A review of the literature suggests that 16S DNA PCR from valve tissue is a more sensitive diagnostic test than culture. It is also extremely specific, based on a sequence match of at least 500 base pairs.

Key Words: *Endocarditis*; S salivarius; *16S DNA PCR*

Une endocardite à culture négative diagnostiquée au moyen de la réaction en chaîne de la polymérase ADN 16S

La réaction en chaîne de la polymérase (PRN) ADN 16S est une technique d'amplification moléculaire qui peut être utilisée pour déterminer les pathogènes bactériens en cas d'endocardite à culture négative. L'ADN bactérien peut être isolé des tissus valvulaires excisés par voie chirurgicale ou du sang recueilli dans des fioles d'EDTA. Cette technique est particulièrement utile pour déterminer le pathogène bactérien dans les cas d'endocardite à culture négative. Les auteurs présentent le cas d'un homme de 48 ans qui a consulté à cause d'une régurgitation aortique importante et d'un prodrome de faible fièvre depuis quatre mois. Les cultures du sang et des tissus valvulaires effectuées après le remplacement valvulaire se sont révélées négatives. Un échantillon de tissu valvulaire a été soumis à une PCR ADN 16S, laquelle a déterminé la présence de *Streptococcus salivarius*, interprété comme le véritable diagnostic. D'après une analyse bibliographique, la PCR ADN 16S prélevée dans le tissu valvulaire serait un test diagnostique plus sensible que la culture. Elle est également d'une extrême spécificité, grâce à un appariement séquentiel d'au moins 500 paires de base.

CASE PRESENTATION

A 48-year-old man presented to a peripheral hospital in November 2010 with a one-month history of chest tightness and shortness of breath. The shortness of breath was present at rest and worsened with activity. He also reported a four-month history of fatigue and intermittent low-grade fever. The patient described a period of respiratory illness with flu-like symptoms shortly after skinning rabbits, six months before the start of his symptoms.

His medical history was significant for gastroesophageal reflux disease as well as an excised stage I melanoma. There was no previous history of ischemic heart disease, left ventricular dysfunction or valvular abnormalities. There was no history of smoking, alcohol use or intravenous drug use.

Physical examination demonstrated normal vital signs and normal jugular venous pressure. He was afebrile. There was a positive 'water-hammer' carotid pulse noted. There was also a loud III/VI diastolic murmur heard along the right sternal border with radiation to the carotid arteries bilaterally. On respiratory examination, there were a few bilateral inspiratory wheezes. The remainder of the physical examination was unremarkable.

Transthoracic echocardiogram revealed 'dilated left heart chambers' with 'preserved left ventricular systolic and diastolic function' and 'severe aortic regurgitation'. The patient was transferred to the cardiac surgery service for evaluation.

An initial complete blood count showed a white blood count of 10.4×10^9/L, hemoglobin level of 158 g/L and platelet count of 205×10^9/L. Electrolytes, urea and creatinine levels were normal. An electrocardiogram revealed a Mobitz type II second-degree atrioventricular block. Chest x-ray showed an enlarged cardiac silhouette with clear lung fields. Repeat echocardiogram revealed 'severe aortic insufficiency through a perforated inferior cusp', with a markedly dilated left ventricle with normal function. There was a large regurgitant orifice measuring 6 mm in diameter through the inferior cusp, and the edges of the cusp were fibrotic with small mobile strands seen. Cardiac catheterization revealed 'severe aortic insufficiency [and an] enlarged left ventricle with well preserved systolic function' and normal coronary artery anatomy.

The patient was started on empirical treatment for bacterial endocarditis with intravenous vancomycin 1 g every 12 h and intravenous gentamicin 90 mg every 8 h. A temporary pacemaker was placed. Blood cultures sent on admission to hospital before antibiotic administration did not yield any growth at 21 days. A urine sample sent for *Legionella pneumophilia* serogroup 1 antigen testing was negative. Serological testing for *Coxiella burnetti* and *Brucella* species was negative. The anti-*Bartonella* titre was 1:128, with a convalescent anti-*Bartonella* titre obtained three months later showing a decreased titre of 1:64.

Six blood cultures drawn during febrile periods in hospital did not yield any growth. Two were drawn the day of admission to hospital, and the other four were drawn during the first week, just before surgery. All cultures were drawn from peripheral sites. Intravenous ciprofloxacin 400 mg every 12 h was subsequently added to the vancomycin and gentamicin regimen (1). The patient underwent an aortic valve replacement with a 25 mm mechanical valve (St Jude Medical, USA) on November 15, 2010. Pathological examination of the aortic valve tissue showed acute inflammation, fibrinoid necrosis, calcifications and fibrodegenerative changes. Gram-stain of the valve tissue

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was negative. Three valve tissue samples were obtained, two for tissue culture via conventional microbiological methods, and a third sample was sent to the molecular laboratory at the Queen Elizabeth II Health Sciences Centre in Halifax, Nova Scotia.

16S DNA polymerase chain reaction (PCR) was performed on the tissue sample. Primers from a commercially available kit were used along with the BLAST online database to determine that DNA from *Streptococcus salivarius* was present in the tissue. The patient was discharged from hospital with an uncomplicated postoperative course, and was prescribed intravenous therapy with ceftriaxone and gentamicin. The patient had one re-admission to hospital one month following discharge for symptomatic bradycardia and was discharged after pacemaker rate adjustment. One year after discharge, he was able to perform strenuous work and has normal prosthetic valve and pacemaker function.

DISCUSSION

Culture-negative endocarditis may cause up to 31% of all cases of infective endocarditis (IE) (2). Our patient had clear echocardiographical evidence of new regurgitation and a history of fever on presentation, but both his blood and tissue cultures were negative. This would satisfy one major and one minor criterion for a diagnosis of possible IE by the modified Duke criteria (1,3). In this setting, empirical antibiotic treatment is appropriate to cover all likely organisms, but the identification of a specific organism is preferred for appropriate medical and surgical management.

It is difficult to achieve a diagnosis of definite IE using the modified Duke criteria if all cultures are negative, although it can be achieved with minor criteria. Molecular diagnostic tests applied to excised tissue have not been included in the Duke criteria to date (1).

Our patient had an interesting history of skinning rabbits, suggesting a diagnosis of *Francisella tularensis*. Tularemia is not commonly associated with endocarditis, rather, it manifests with adenopathy, pharyngitis or pneumonia. If it were the correct diagnosis, bacterial DNA from *Francisella* would have been detected by 16S PCR.

Serology for *Bartonella* species was positive; however, because the titre was declining over time, and because Bartonella endocarditis is generally associated with a very high titre (greater than 1:800) (4,5), this was interpreted as a past exposure and not an acute infection, according to guidelines from the National Microbiology Laboratory (Winnipeg, Manitoba) where the test was performed.

The identification of *S salivarius* DNA from the valve tissue was considered the true diagnosis based on the propensity of this organism to cause IE, the lack of evidence for the diagnosis of other causes of culture-negative endocarditis and the lack of history of previous endocarditis.

The 16S DNA gene contains six conserved sequences that all bacteria have in common, as well as variable regions unique to certain bacterial species (6). PCR is used to amplify this variable region and the amplicon was sequenced and matched to an Internet database of 16S sequences. This technique can be applied to peripheral blood or valve tissue, and it can be performed in one day.

A recent, large prospective case series involving 819 suspected cases of blood culture-negative endocarditis demonstrated that the best histological, serological and molecular testing available was still only able to identify a causative microorganism in 62.7% of cases (2). 16S PCR was performed on 227 valve tissue samples and was positive in 150 (sensitivity 66%). PCR was able to make a diagnosis in 109 cases in which serological tests were not diagnostic. Of these 109 cases, 106 were tested with PCR from valve tissue and only three were tested with PCR from peripheral blood, demonstrating that PCR from peripheral blood only rarely contributes additional diagnostic information. Because broad-range PCR provides a bacterial DNA sequence of 500 base pairs or longer, a perfect match at all 500 positions with a sequence from a database provides outstanding specificity.

A recent, retrospective case series involving 269 valve tissues removed from 241 patients with IE diagnosed using the Duke criteria amplified 16S DNA and 23S DNA targets (7). Among patients with proven IE, 16S PCR was positive in 149 of 185 tissue samples (80.5% sensitivity), with no positivity found among cases of healed IE, ambiguous IE or no IE (100% specificity). Valve tissue culture demonstrated sensitivity of 33.4% and specificity of 96.6%.

In a retrospective collection involving 52 tissue samples taken from resected heart valves from patients suspected of having IE based on clinical, microbiological and echocardiograhical criteria, sensitivity of PCR was 42% compared with 7.8% for valve tissue culture and 11.8% for tissue Gram-staining (8). Another study involving 74 heart valves used blood culture as a reference standard, which is clearly a biased standard for diagnosis of culture-negative endocarditis. PCR demonstrated a sensitivity of 72% compared with valve tissue culture at 26% (9). Sensitivity of valve tissue culture was markedly reduced in patients who had received antibiotic therapy for five days or more before surgery. This is a significant limitation, because in most cases of IE, surgery would occur later than five days after the initiation of antibiotics.

Because patients with IE often receive empirical antibiotics before blood culture collection, PCR, in addition to conventional diagnostic testing, may offer an advantage in cases requiring surgery because bacterial DNA may persist after viable organisms are lost. *Streptococcus pneumoniae* DNA may persist seven years beyond the initial episode of endocarditis (10).

Moreover, 16S DNA PCR from tissue may detect species that are difficult to culture. These include *Legionella* species, *Bartonella* species, *Chlamydia* species, *Tropheryma whippelii*, *C burnetii* and *Mycobacterium* species. Timely identification of rare or fastidious microorganisms in the setting of acute infection is significantly more likely with the PCR technique. In the Fournier series of 106 blood culture-negative valve tissues diagnosed by PCR, 30 detected *C burnetii*, 26 detected *Bartonella* species and 17 detected *T whippelii* (2).

Disadvantages of 16S DNA PCR with heart valve tissue include false negatives due to PCR inhibitors in the tissue and potential environmental contamination with nonpathogenic bacterial DNA. The test is expensive, and no susceptibility information is provided. The identification is as good as the quality of the database used to search the sequence. The best available DNA match in the database is often considered the true diagnosis even though the true diagnosis may not be present in the database. For this reason, a larger sequence database containing only high-quality, peer-reviewed submissions will provide optimal specificity. Few reports have compared sequences searched with different databases (11).

The present case joins a number of reported cases in which molecular techniques provided the only means of diagnosis for culture-negative endocarditis (12-14). When tissue is available, even after prolonged antibiotic therapy, broad-range PCR may provide diagnoses that are not possible with serological or conventional culture means.

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