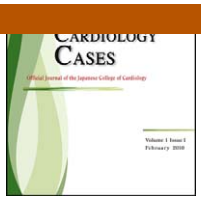
Journal of Cardiology Cases (2011) 3, e82–e85



ScienceDirect
journal homepage: www.elsevier.com/locate/jccase



Case Report

# Culture negative mitral valve endocarditis caused by *Neisseria gonorrhoeae* confirmed by 16S rDNA sequence analysis of resected valvular tissue

**Stuart J. Butterly (MD)[a,*], David F.M. Looke (MD)[b,c], Shane Byrne (BSc)[d], Gerry Kaye (MD)[a,c]**

[a] *Department of Cardiology, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Queensland 4102, Australia*
[b] *Department of Infectious Diseases and Clinical Microbiology, Princess Alexandra Hospital, Brisbane, Australia*
[c] *The University of Queensland, Brisbane, Australia*
[d] *Molecular Pathology, Sullivan Nicolaides Pathology, Brisbane, Australia*

Received 12 July 2010; received in revised form 6 December 2010; accepted 24 January 2011

**KEYWORDS**
Endocarditis;
Polymerase chain reaction;
*Neisseria gonorrhoeae*;
16S DNA

**Summary** Infective endocarditis is a dynamic disease with changing epidemiology and diagnostic strategies. Culture negative endocarditis poses a particular problem for clinicians regarding appropriate antimicrobial therapy and adequate duration of therapy. Utilization of nucleic acid amplification techniques and subsequent sequencing has provided clinicians an alternative to traditional phenotypic microbial identification which has been extremely useful in identification of fastidious organisms. We report a case of a young male with culture negative native mitral valve endocarditis and subsequent 16S rDNA sequencing of *Neisseria gonorrhoeae* from excised valvular tissue and embolic material. Identification of this organism with novel nucleic acid amplification and 16S rDNA sequence analysis techniques permitted targeted antibiotic therapy and successful treatment of this potentially fatal disease.


## Introduction

Infective endocarditis is a disease that is forever evolving with frequent changes in microbiological profile and clinical scenario. Along with this change in disease epidemiology have been advances in diagnosis and therapy. We report the case here of a young man with culture negative native mitral valve endocarditis with subsequent microbiological identification by 16S rDNA sequencing allowing targeted antimicrobial therapy.

## Case report

A 26-year-old male, without any significant medical history, presented to the emergency department with respiratory

* Corresponding author. Tel.: +61 7 3176 2111; fax: +61 7 3176 5640.
*E-mail address:* stuart_butterly@health.qld.gov.au (S.J. Butterly).

1878-5409/$ – see front matter 
doi:10.1016/j.jccase.2011.01.007

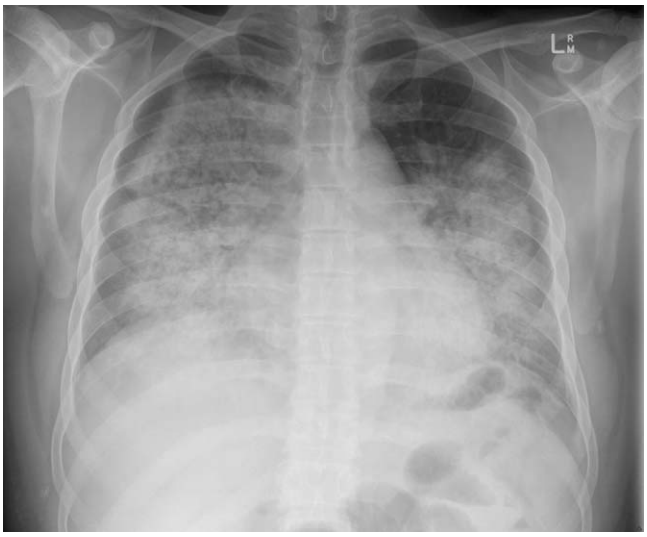


**Figure 1** Chest X-ray at admission.

distress. He had a suffered a productive cough over the previous 6 weeks, with progressive dyspnea and orthopnea for the last 10 days. On the day of admission he had an episode of chest tightness and haemoptysis. He had not undergone any recent dental procedures and had not received antimicrobial therapy prior to presentation.

On examination he was distressed with respiratory accessory muscle use and unable to lay flat. His temperature was 39.6 °C, heart rate 120/min, respiratory rate 30/min, blood pressure 130/79 mmHg with an oxygen saturation of 82% on room air. His jugular venous pressure was elevated by 3 cm. There were no audible murmurs but a third heart sound was present. Coarse crackles were present bilaterally throughout hypoventilated lung fields.

Investigations revealed a haemoglobin of 8.8 g/dL (88 g/L), a white cell count of $16.4 \times 10^3/\mu L$ ($16.4 \times 10^9/L$), predominantly a neutrophilia (neutrophil count $14.17 \times 10^3/\mu L$ ($14.17 \times 10^9/L$)). Platelet count was $320 \times 10^3/\mu L$ ($320 \times 10^9/L$). Liver function tests (transaminases) were mildly elevated. Arterial blood gas analysis revealed an acute respiratory alkalosis (pH 7.51, $pO_2$ 57 mmHg, $pCO_2$ 27 mmHg, $HCO_3$ 21 mEq/L (21 mmol/L)). The erythrocyte sedimentation rate was >100 mm/h and C-reactive protein was 16.9 mg/dL (169 mg/L). Six sets of blood cultures were collected and incubated for a minimum of 5 days in the Bac-T alert blood culture system. Midstream urine collection revealed $>500 \times 10^6$/L leukocytes and $>500 \times 10^6$/L red blood cells. Human immunodeficiency virus testing was negative. There was no bacterial growth after 24 h of urine culture. All blood cultures remained negative throughout admission.

Chest X-ray revealed a spontaneous pneumothorax, bilateral pulmonary infiltrates and fluid in the horizontal fissure (Fig. 1). An intercostal catheter was placed in the hemithorax — air and hemoserous fluid was aspirated. A diagnosis of community-acquired pneumonia was made and intravenous benzylpenicillin (1200 mg QID), gentamicin (5 mg/kg daily then dosed on 12 h plasma concentration according to the Begg Nomogram), and azithromycin (500 mg OD) were commenced after blood cultures were taken.

Over the following 24 h he developed progressive hemodynamic decline necessitating intubation, ventilation, and transfer to the intensive care unit. Antibiotic therapy was changed to vancomycin (1000 mg BD), gentamicin, and ceftriaxone (2000 mg BD), and dobutamine commenced for blood pressure support.

Thirty-six hours after admission transthoracic echocardiography demonstrated a 1.7 cm vegetation attached to the posterior leaflet of the mitral valve and severe mitral regurgitation (Fig. 2). Initial urgent attempts to repair his mitral valve were unsuccessful and it was subsequently replaced with a 29 mm Perimount bioprothetic valve. Mitral valve tissue histology demonstrated features of bacterial endocarditis but no organisms were identified on Gram stain and tissue culture was negative.

He was extubated and returned to the ward, continuing on the same antimicrobial regimen, but continued to have intermittent low grade fevers. On the 14th postoperative day he developed acute back pain. Abdominal computed tomography imaging revealed a 3.5 cm mycotic aneurysm of the superior mesenteric artery (SMA). Transesophageal echocardiography demonstrated normal native and prosthetic valvular function and no vegetations. The SMA aneurysm was surgically ligated and tissue sent for histology and microbiology.

As cultures of blood, valvular, and aneurysm tissue were negative, molecular testing of the mitral valve and mycotic aneurysm tissue by amplifying the 16S rRNA gene was carried out using 4 primer pairs (fD1 mod/16S1RR-B, 63F/16S1RR-B, 8F2/806R, and 515F/13R) [1]. The resulting polymerase chain reaction (PCR) products were sequenced and the sequence data submitted for comparison to data held in the GenBank NR sequence database using the Basic Local Alignment Search Tool (BLASTN) algorithm [2]. The sequences obtained from the PCR products demonstrated 99% homology with 16S rRNA gene sequences from *Neisseria gonorrhoeae*.

The patient continued intravenous ceftriaxone (2000 mg daily) for a further 4 weeks to complete a total of 7 weeks of antimicrobial therapy. Further inquiry revealed that the patient had unprotected sexual intercourse with a female 5 months prior to his acute presentation. At 2 years he remains well and has suffered no further complications.

## Discussion

Infective endocarditis remains a significant medical issue due mainly to its associated mortality and economic burden. Infective endocarditis is usually diagnosed by a combination of clinical, microbiological, and histological features according to the Duke Criteria [3].

New guidelines for the prevention, diagnosis, and treatment of infective endocarditis have recently been released [4]. These guidelines recommend classification on the basis of microbiological findings in addition to the clinical scenario. Four categories have been proposed: Infective endocarditis with positive blood cultures; infective endocarditis with negative blood cultures because of previous antibiotics; infective endocarditis with frequently negative blood cultures; and infective endocarditis with constantly negative blood cultures [4]. The last three groups would pre-

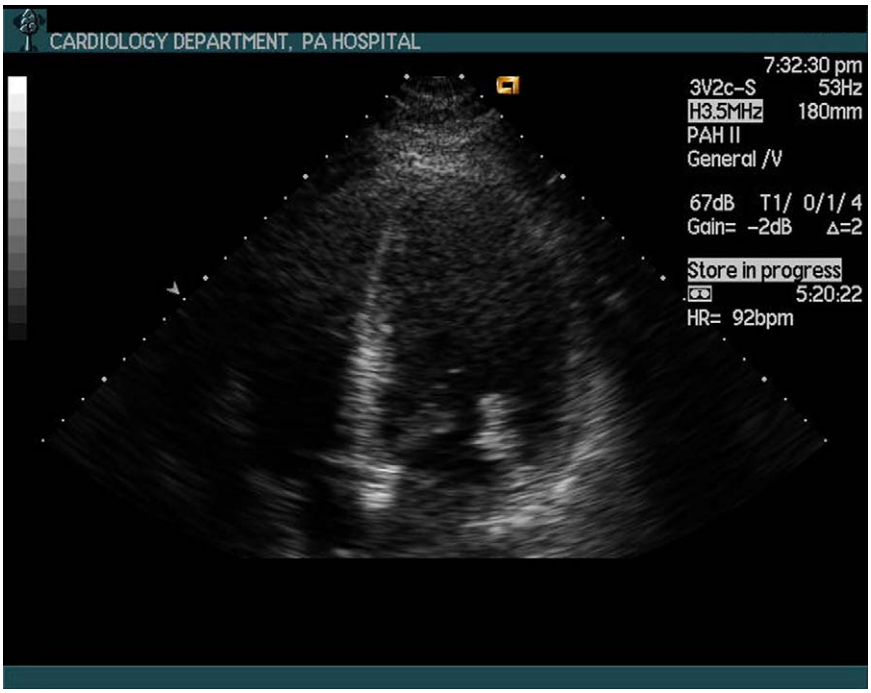



**Figure 2** Transthoracic echocardiogram: apical 4-chamber view demonstrating vegetation attached to the posterior mitral valve leaflet.

viously have been considered culture-negative-endocarditis and a positive diagnosis usually requires serological testing or gene amplification. Paradoxically, improved antimicrobial regimens may have increased diagnostic failure as micro-organisms present on excised tissue are non-viable [5].

The PCR is a molecular biology technique utilized to amplify small quantities of DNA exponentially. The method uses primers (complimentary DNA fragments; in this case targeting part of the 16S rRNA gene), heat stable DNA polymerase, and repeat thermal cycling to allow melting and replication of the DNA fragments of interest.

PCR allows rapid and reliable detection of fastidious and non-culturable agents in patients with infective endocarditis [6] and has been validated using valve tissue from patients undergoing surgery for infective endocarditis [6]. In particular, identification of microorganisms by 16S rRNA gene sequencing has emerged as a more objective, accurate, and reliable method of bacterial identification when compared to phenotypic identification methods (Gram stain results, colony morphology, growth requirements, and enzymatic/metabolic activity) [4]. The sensitivity and specificity of PCR has been reported to be up to 72% and 100% respectively while tissue culture has sensitivity of 23% and specificity of 62% [7]. Valvular tissue PCR has been shown to provide a positive result when tissue culture remains negative and its sensitivity is independent of the duration of pre-surgery antibiotic therapy [7].

The lack of reliable application to whole blood samples (surgical specimens are better), risk of sample contamination, risk of contamination with endogenous reagent derived bacterial DNA, and false negatives due to inhibitors are limitations of 16S rDNA PCR. Laboratory sequence database comprehensiveness and intra- or inter- species variation also impacts on PCR utility [7]. Fungal endocarditis is not identified from 16S rRNA gene sequencing and PCR is unable to provide antibiotic sensitivities of the identified organism. Some studies report persistently positive PCR results months after eradication of the infection potentially confounding the diagnosis [8]. It is recommended that PCR of excised valve tissue or embolic material be performed in patients with negative blood cultures who undergo valve surgery or embolectomy [4].

Gonococcal endocarditis was relatively common in the pre-antibiotic era with 11—26% of cases being due to *N. gonorrhoeae* [9]. Disseminated infection occurs in 1—3% of all gonococcal infections and endocarditis is a complication in 1—2% of these patients [9]. Mortality of up to 19% occurs despite appropriate medical and surgical treatment [9]. The diagnosis is usually established by successful culture of *Neiserria gonorrhoeae* from peripheral blood or from surgically sampled valvular tissue [9] however, these tests may remain negative for some time or positive results may never be achieved due to pre-treatment with antibiotics. Current Australian empirical antibiotic treatment for infective endocarditis (after three sets of blood cultures) includes benzylpenicillin, fluxcloxacillin, and gentamicin and does not provide adequate cover for the increasing frequency of penicillin-resistant gonococcal organisms encountered in clinical practice [10]. Delays to adequate treatment can have significant consequences including death.

Recently, *N. gonorrhoeae* endocarditis confirmed by nucleic acid amplification performed on aortic valve tissue has been reported [6]. The authors propose that nucleic acid amplification assays provide a rapid method of diagnosing *N. gonorrhoeae* endocarditis or other suspected disseminated gonococcal disease.

In this case, culture of blood and valvular tissue failed to yield a positive result, however, PCR of excised tissue

was able to establish the organism involved. The patient's antibiotic regimen was adjusted prior to the organism identification and fortuitously included cover for *N. gonorrhoeae*, thus he received targeted antibiotic therapy early in his hospital admission. Had he continued on empirical antibiotics as recommended by Australian guidelines [10], adequate therapy would not have been initiated until the PCR results were available. PCR of valvular and mycotic aneurysm tissue with subsequent organism identification allowed for confident duration of antibiotic therapy to be established in this patient.

In the case of organisms resistant to empiric antibiotic therapy, early identification is imperative to avoid adverse outcomes for the patient. Early PCR confirmation of gonococcal endocarditis from excised valvular tissue can provide support for targeted treatment in this aggressive, potentially fatal disease.

## References

[1] Nikkari S, Lopez FA, Lepp PW, Cieslak PR, Ladd-Wilson S, Passaro D, Danila R, Relman DA. Broad-range bacterial detection and the analysis of unexplained death and critical illness. Emerg Infect Dis 2002;8:188—94.

[2] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389—402.

[3] Durack DT, Lukes AS, Bright DK. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings: Duke Endocarditis Service. Am J Med 1994;96:200—9.

[4] Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I, Moreillon P, de Jesus Antunes M, Thilen U, Lekakis J, Lengyel M, Müller L, Naber CK, Nihoyannopoulos P, Moritz A, et al. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC), Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J 2009;30:2369—413.

[5] Breitkopf C, Hammel D, Scheld H, Peters G, Becker K. Impact of a molecular approach to improve the microbiological diagnosis of infective heart valve endocarditis. Circulation 2005;111:1415—21.

[6] Nielsen U, Knudsen J, Pedersen N, Møller J. *Neisseria gonorrhoeae* endocarditis confirmed by nuleic acid amplification assays performed on aortic valve tissue. J Clin Microbiol 2009;47:865—7.

[7] Voldstedlund M, Nørum Pedersen L, Baandrup U, Klaaborg K, Fuursted K. Broad range PCR and sequencing in routine diagnosis of infective endocarditis. APMIS 2008;116:190—8.

[8] Branger S, Casalta JP, Habib G, Collard F, Raoult D. *Streptococcus pneumoniae* endocarditis: persistence of DNA on heart valve material 7 years after infectious episode. J Clin Microbiol 2003;41:4435—7.

[9] Jackman Jr JD, Glamann DB. Gonococcal endocarditis: twenty-five year experience. Am J Med Sci 1991;301:221—30.

[10] Antibiotic Guidelines: Cardiovascular System Infections: Endocarditis: empirical treatment [revised 2006 June]. In: eTG complete [Internet]. Melbourne: Therapeutic Guidelines Limited; 2009 November. https://online-tg-org-au.cknservices.dotsec.com/ip/tgc/abg/4562.htm (accessed 8.01.10).