血流动力学上急需主动脉瓣替换。患者接受了主动脉瓣替换术和二尖瓣置换术。血培养未能育出致病菌。血液学检查显示白细胞计数7.2×10⁹/L，C反应蛋白33 mg/L，血红蛋白92 g/L和肌酐151 μmol/L。他被给予静脉高剂量阿莫西林，氯霉素和庆大霉素治疗，临床状况明显改善。

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**ABSTRACT**

Blood culture negative endocarditis (BCNE) accounts for up to 20% of infective endocarditis. While the most common cause of BCNE remains the initiation of antibiotics prior to culture, intracellular organisms such as *Coxiella* and *Bartonella* spp account for a significant proportion of cases. Identifying the infecting organism remains important to ensure optimal antimicrobial treatment. However, these organisms can be difficult to diagnose. We outline a systematic approach to BCNE. Over half of patients with infective endocarditis now undergo early surgery and 16S ribosomal ribonucleic acid (rRNA) polymerase chain reaction (PCR) analysis of excised tissue can be vitally important to secure a diagnosis. Molecular testing is likely to become a key tool in improving outcomes from BCNE and contribute to an improved understanding of the aetiology. We advocate modifying the Duke criteria to incorporate organisms identified on molecular testing, including 16S rRNA PCR, in particular from explanted tissue.

**KEYWORDS:** Endocarditis, culture negative, 16S rRNA, PCR, *Bartonella henselae*

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**Case presentation**

一个34岁的男性，作为配送司机，通常工作状况良好，伴有两周的病史，表现为运动时的气促和干咳。他有发热，心前区明显收缩期杂音，伴有严重主动脉瓣反流和肺部充血。没有外周的特征性体征。血液学检查显示白细胞计数7.2×10⁹/L，C反应蛋白33 mg/L，血红蛋白92 g/L和肌酐151 μmol/L。他被给予利尿剂和胸壁超声心动图显示一个二叶主动脉瓣，伴有严重主动脉瓣反流和几个植被，大小约1.8 cm。患者被给予静脉高剂量阿莫西林，氯霉素和庆大霉素治疗，临床状况明显改善。

在英国，血流动力学上急需主动脉瓣替换的患者中，超过一半会在早期手术。16S rRNA聚合酶链反应（PCR）分析从切除的组织中可以非常关键地确认诊断。分子测试有可能会成为改善BCNE结果的关键工具，并有助于更好地理解病因。我们建议将杜克标准修改为在分子测试中识别到的病原体，包括16S rRNA PCR，特别是在从摘除组织中。

**Introduction**

尽管医学上的进步，但血流动力学上急需主动脉瓣替换的感染性心内膜炎的发病率仍有显著增长。在英国，血流动力学上急需主动脉瓣替换的患者中，超过一半会在早期手术。分子测试（PCR）分析从切除的组织中可以非常关键地确认诊断。分子测试有可能会成为改善BCNE结果的关键工具，并有助于更好地理解病因。我们建议将杜克标准修改为在分子测试中识别到的病原体，包括16S rRNA PCR，特别是在从摘除组织中。
We suggest the following pragmatic approach to determining the causative organism, in line with current guidelines and evidence (Fig 1). History taking may identify recognised host and epidemiological factors associated with different aetiologies of BCNE (Table 2). Determining these factors, including immune status e.g. HIV status informs further diagnostic testing.

Serology

Serology for Coxiella burnetii is first line in the investigation of BCNE; Coxiella phase 1 immunoglobulin G (IgG) ≥800 is diagnostic of Coxiella burnetii infection and qualifies as a major Duke criterion.7 Although Bartonella spp IgG ≥800 is diagnostic for Bartonella spp infection, serological testing as stated is not currently available in the UK.28 In countries where serology is available, it should be recognised that cross-reactivity occurs between Bartonella spp and Coxiella burnetii and it is notoriously difficult to interpret serology in this setting.14 The British Society for Antimicrobial Chemotherapy (BSAC) advise considering Brucella serology in the setting of dietary or travel-related exposure (Table 2).26 Mycoplasma, Legionella and Mycobacteria endocarditis are exceptionally rare.17,25 Routine serological testing for Legionella, Mycoplasma and Chlamydia spp is not recommended.26 Previous serological Chlamydia spp diagnoses may reflect cross-reactivity with Bartonella spp (with no valve positives to date).29,30 In two large studies evaluating a range of serological tests in BCNE, 624 patients were diagnosed by serology (from a total of 1,093), and only four cases BCNE would have been missed if serology testing had been restricted to testing for Coxiella burnetii and Bartonella spp only.15,17,31

Examination of explanted tissue

Explanted valve tissue or embolectomy material is routinely sent for microscopy and culture, in part to guide duration of postoperative therapy (with a longer duration of postoperative antibiotic therapy recommended if the organism is grown from the valve).32
Table 2. Pathogens causing blood culture negative endocarditis

<table>
<thead>
<tr>
<th>Organism</th>
<th>Risk factors for infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coxiella burnetii (intracellular)</td>
<td>Occupational exposure to farm animals (sheep, cattle, goats)</td>
</tr>
<tr>
<td>26–37% of BCNE</td>
<td>Living downwind from farms and infected farm material (straw manure)</td>
</tr>
<tr>
<td></td>
<td>Abattoir workers</td>
</tr>
<tr>
<td></td>
<td>Laboratory exposure to pathogens</td>
</tr>
<tr>
<td></td>
<td>Immunosuppression possibly including HIV</td>
</tr>
<tr>
<td>Bartonella henselae (intracellular)</td>
<td>Contact with cats (higher bacteraemia in kittens)</td>
</tr>
<tr>
<td>Bartonella quintana (intracellular)</td>
<td>Contact with human body lice / homeless shelters</td>
</tr>
<tr>
<td>Bartonella spp collectively 12–28% of BCNE</td>
<td>Chronic alcoholism</td>
</tr>
<tr>
<td></td>
<td>Travel to north Africa</td>
</tr>
<tr>
<td>Brucella (fastidious Gram-negative bacilli)</td>
<td>Occupational exposure to farm animals (sheep, cattle, goats)</td>
</tr>
<tr>
<td>Only in endemic areas</td>
<td>Ingesting unpasteurised milk/cheese / undercooked meat</td>
</tr>
<tr>
<td>Fungi</td>
<td>Travel to Middle East</td>
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<tr>
<td>1–2% of BCNE</td>
<td>HIV positive</td>
</tr>
<tr>
<td></td>
<td>Indwelling venous catheter</td>
</tr>
<tr>
<td></td>
<td>Prior cardiac surgery</td>
</tr>
<tr>
<td>Tropheryma whippelii</td>
<td>Occupational exposure to soil / farm animals</td>
</tr>
<tr>
<td>Up to 6% of BCNE</td>
<td>Tuberculosis contact or exposure</td>
</tr>
<tr>
<td>Tuberculosis and other mycobacteria</td>
<td>Mycobacterium chimaera following cardiac surgery</td>
</tr>
</tbody>
</table>

**BCNE** = blood culture negative endocarditis

Histopathological examination of the valve can be valuable. Specific culture media or histopathological stains can be used to look for *Mycobacteria* spp (alcohol and acid-fast bacilli), *Tropheryma whippelii* (periodic acid-Schiff positive macrophages), fungi (hyphae / silver stain) and *Bartonella* spp (Warthin–Starry stain).[^14]

**Molecular**

Serum testing with PCR is not recommended due to low sensitivity and no additional cases of infection were diagnosed following initial serology in a large study.[^17] Molecular techniques to diagnose endocarditis from explanted tissue have been available for over 20 years and are becoming increasingly important in the diagnostic workup of endocarditis.[^30] Currently they remain excluded from the modified Duke criteria. One molecular technique, the pan-bacterial 16S rRNA PCR, uses PCR on homogenised tissue to amplify bacterial genetic material from the 16S rRNA. This genetic material is conserved in all bacteria and hypervariable segments can allow for further identification of bacteria down to a species level. The technique is highly sensitive and specific for identifying the causative organism although clinical correlation is required and it is important to avoid cross-contamination of tissue. Microbial deoxyribonucleic acid can persist for months following infection and the presence of bacteria from PCR analysis does not necessarily imply ongoing infection.

The technique detects the causative organism in the majority of BCNE cases and represents a major advance in the management of cases of endocarditis where antibiotics were given prior to culture, in patients with equivocal serological results, in cases where culture and serology have been negative or, as in the case illustrated, where serological testing is unavailable.[^17][^33] Furthermore, and importantly, molecular sequencing offers improved understanding of the true aetiology of endocarditis in different countries and it is a major advance in the diagnosis and management of this devastating disease.[^34][^35] Modification of the Duke criteria to include tissue PCR has been acknowledged in the latest update from the British Society for Antimicrobial Chemotherapy; however, this has not been formalised.[^26][^36] Current international guidelines on endocarditis reference Duke criteria, excluding molecular testing.[^1]

Fig 1. A pragmatic approach to determining the causative organism in suspected endocarditis. ANA = anti-nuclear antibody; anti-dsDNA = anti-double-stranded deoxyribonucleic acid; BSAC = British Society for Antimicrobial Chemotherapy; ESC = European Society of Cardiology; PCR = polymerase chain reaction; RF = rheumatoid factor; rRNA = ribosomal ribonucleic acid.
**Blood culture negative endocarditis**

**Take home messages**

Endocarditis remains challenging to diagnose and manage as it is associated with a wide diversity of presentation and variable clinical course. The incidence of endocarditis is increasing, particularly in the elderly. All clinicians should have a low threshold to suspect the diagnosis in the setting of unexplained sepsis, a murmur and/or evidence of systemic embolisation particularly in those at increased risk such as native valve disease, prosthetic valve replacement or the presence of an intracardiac device.

The case highlights the need to act quickly when the patient is acutely unwell. Recognition of severe aortic regurgitation prompted early discussion with the endocarditis team and urgent cardiac surgery. Over recent years, there has been increasing recognition of the importance of an experienced endocarditis team perspective on tailored medical, surgical and antimicrobial management. Patients should be discussed urgently if there is haemodynamic instability, heart failure, recurrent systemic embolisation or uncontrolled infection.

A significant proportion of endocarditis is blood culture negative. This review highlights a pragmatic approach to these patients. In the vignette, BCNE was caused by *Bartonella henselae* infection and diagnosis of the causative organism was delayed by the current absence of serological testing in the UK. Although with hindsight there were epidemiological clues when an extended history was taken, 16S rRNA PCR analysis of tissue from the explanted valve was ultimately diagnostic and changed antimicrobial management.

> Consider endocarditis early.
> Management of endocarditis with a multidisciplinary endocarditis team improves outcome through individualised care and early surgery.
> Blood culture negative endocarditis is common. In patients who undergo surgery, excised tissue should be sent for 16S rRNA PCR. This can diagnose the causative organism and will inform optimal antimicrobial treatment and duration. This is of increasing relevance given that early surgical intervention is associated with a lower risk of mortality in infective endocarditis, and over 55% of patients undergo surgery early in their clinical course.
> We advocate modifying the Duke criteria to include organisms detected on explanted tissue using molecular testing. This valuable test is likely to become a key tool in improving outcomes from BCNE and better understanding the aetiology.

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**References**

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Rebuilding the NHS
RCP priorities for the resetting of services

The RCP has set out nine key priorities for the resetting and rebuilding of NHS services, now that the initial COVID-19 peak has passed.

The publication covers the role of the medical specialties and emphasises that our healthcare system must not simply return to how it was pre-pandemic. From routine care to reducing health inequalities, particularly for people from ethnic minority backgrounds, we have the opportunity to embed long-term improvements.

Download the document:
www.rcplondon.ac.uk/rebuilding-the-NHS