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REVIEW

Laboratory Approach to the Diagnosis of Culture-Negative Infective Endocarditis

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Blood-culture negative endocarditis (BCNE) accounts for up to 35% of all cases of infective endocarditis (IE), a serious life-threatening condition with considerable morbidity and mortality. Rapid detection and identification of the causative pathogen is essential for timely, directed therapy. BCNE presents a diagnostic and therapeutic challenge. Causes of BCNE are varied including: prior treatment with antibiotic agents prior to blood culture collection; sub-optimal specimen collection; and/or infection due to fastidious (eg. nutritionally variant streptococci), intracellular (eg. *Coxiella burnetii*, *Bartonella* species) or non-culturable or difficult to culture organisms (eg. *Mycobacteria*, *Tropheryma whippelii* and fungi); as well as non-infective aetiologies. Here, we review aetiological and diagnostic approaches to BCNE including newer molecular based techniques, with a brief summary of imaging investigation and treatment principles.

Keywords infective endocarditis • blood culture negative endocarditis • nuclear amplification tests • device associated infections • Q fever • endocarditis

Introduction

Infective endocarditis (IE) is a serious, life-threatening condition associated with significant morbidity and mortality [1–3]. Rapid detection and identification of the causative pathogen is essential in ensuring timely and directed therapy. Diagnosis of IE is usually made by a combination of clinical, echocardiographic, histological and microbiological criteria as set out in the modified Duke's criteria [2,4]. However, IE, whereby no causative microorganism is grown from blood culture or from diseased cardiac tissue by standard laboratory methods, may occur. ''Blood culture negative endocarditis'' (BCNE) [3,5] which accounts for 2.1–35% of all IE cases remains a diagnostic and therapeutic challenge [5–7].

The causes of BCNE are varied. Although receipt of antibiotic agents prior to blood culture collection is the most common cause (35–40%), sub-optimal specimen collection and/or infection due to fastidious, intracellular or non-culturable organisms are other causes of BCNE [5,6]. In addition, non-infective aetiologies such as marantic endocarditis and those in the setting of autoimmune diseases such as lupus are also included in the umbrella of BCNE [3,5,6]. Diagnostic approaches to BCNE include both culture-, and non-culture based methods including the use of serological tests and of molecular techniques such as those that employ broad range polymerase chain reaction (PCR) assays on affected heart valves and other cardiac tissue [2,3,6,8]. To this end, liaison between the physician and the microbiology and tissue pathology laboratory is critical to ensure appropriate diagnostic investigations are performed. Here we review the aetiology, and microbiological diagnostic approaches including newer molecular based techniques of BCNE, with a brief summary of imaging investigation and treatment principles.

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Aetiology

There are a numerous causes of BCNE resulting from infection with organisms that are either difficult to grow or which are non-culturable. Typically, in patients presenting with suspected IE, at least three sets of blood cultures (including an aerobic and anaerobic bottle in each set) from separate venipuncture sites should be obtained, with the first and last samples drawn at least one hour apart [2,9]. The more common microbial aetiologies of BCNE (see Table 1) [2,3,5] include intracellular pathogens such as *Coxiella burnetti*, *Bartonella*, *Legionella*, *Mycoplasma* and *Chlamydia* species [6]. Non-culturable organisms, or organisms that are difficult to culture are best exemplified by *Tropheryma whippleii*, fungi and mycobacterial species, especially non-tuberculous mycobacteria and less commonly *Mycobacterium tuberculosis* [5,6]. Fastidious organisms may also pose a problem in yielding a positive blood culture and these include members of the HACEK group (*Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species) and nutritionally variant streptococci. In one study, where comprehensive serologic, molecular and histopathological methods were used to investigate the cause of BCNE, the causative agent was found only in 62.7% of patients [5].

Intracellular Pathogens

Coxiella burnetii

Q9 *Coxiella burnetii* is an obligate intracellular pathogen and causes Q fever. It is found worldwide except in New Zealand [10,11]. In acute infection, Q fever has protean manifestations and may include febrile illness with myalgia, headaches and hepatitis, often mistaken for a viral infection. If untreated, Q fever can have fatal consequences [10,12] and lead to the chronic phase, most often manifesting as BCNE, however, osteomyelitis and granulomatous hepatitis can also occur. Patients with Q fever endocarditis frequently have minimal valvular changes seen on echocardiography [12].

Serology is the mainstay of diagnosis of Q fever endocarditis (see, Laboratory Diagnosis: General). Culture of *C. burnetii* from blood cultures or affected heart valves may be attempted in specialised laboratories, and is not routinely performed in the diagnostic laboratory [13]. Immunohistochemistry on resected heart valves and special Gimenez stain is often used to aid histological diagnosis of *C. burnetii*-infected tissues [6]. The presence of doughnut granulomas in histological sections are highly suggestive of Q fever endocarditis. Molecular methods for diagnosis include broad Q10 range PCR or *C. burnetii*-specific PCR on freshly excised heart valve tissue [6,14,15]. Treatment of Q fever endocarditis is listed in Table 1.

Bartonella Species

Bartonella spp. are small intracellular Gram-negative bacteria that cause a range of infections in immunocompetent and immunocompromised hosts. *Bartonella henselae* is transmitted to humans by a cat scratch or bite or by cat fleas whilst *B. quintana* is transmitted by the human body louse [13,16]. *Bartonella henselae* typically causes cat scratch disease, bacillary angiomatosis and hepatic peliosis in human immunodeficiency virus (HIV) infected patients whilst *B. quintana* causes trench fever, lymphadenopathy with fever, and bacillary angiomatosis. Both species, and rarely other species *B. elizabethae* and *B. vinsonii*, can cause endocarditis [6,8,17].

Patients with *Bartonella* endocarditis usually have negative blood cultures and diagnosis is made using a combination of serology, PCR/DNA sequencing and histological examination of resected heart valve tissue [6,15] (see also Laboratory Diagnosis below). Cell culture-based methods are required to isolate the organism from heart valves or blood cultures and hence are not readily adaptable to the diagnostic laboratory [13]. Table 1 summarises the treatment of *Bartonella* endocarditis.

Legionella Species

Legionella species cause pneumonia and Legionnaires' disease but rarely extra-pulmonary infections including endocarditis, myocarditis, peritonitis or pyelonephritis [13]. To date, only 19 cases of endocarditis due to *Legionella* species have been reported [6,18–20]. Patients often present with chronic symptoms of low-grade fever, weight loss, malaise, night sweats and symptoms of congestive cardiac failure. Echocardiography may not reveal distinct vegetation, similar to patients with Q fever endocarditis [6,19,21].

Endocarditis due to *Legionella* species should be suspected in cases of BCNE where serological tests for Q fever and *Bartonella* return negative. Endocarditis due to *Legionella* species can be nosocomial in origin following cardiothoracic surgery and diagnosis may take months or years [18,21]. Community-acquired cases have had a preceding history of pneumonia and the diagnosis is often made during that admission. As before, diagnosis can be made by one or more of cultures of the resected valve or periodic subculture of the incubating blood culture broth onto specialised media supportive for *Legionella* [6,13], molecular methods and serology [6,19].

Chlamydia Species

Chlamydia species are a rare cause of BCNE. To date, approximately 15 cases have been reported [22–26]. Because *Chlamydia* species are obligate intracellular organisms, they can only be grown on tissue culture. As a result, serological diagnostic methods are the mainstay of diagnosis (see Laboratory Diagnosis: Serology). This approach, however, is problematic as *Chlamydia* antigens often cross-react with those of other microorganisms including *Bartonella* spp [6]. Because most early case reports of *Chlamydia* endocarditis were diagnosed by serological tests, the certainty of diagnosis can be questioned [23,25] Other diagnostic methods include identification of the organism by using immunohistochemistry in resected valves or by molecular methods [6,22].

Mycoplasma Species

Mycoplasma species are also rare causes of BCNE with nine cases reported to date; 8/9 were due to *Mycoplasma hominis* and 1/9 due to *Mycoplasma pneumonia* [27–29]. All but one

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