CASE REPORT

Culture-negative endocarditis due to *Bartonella quintana*

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Bartonella spp. was first described as a possible cause of culture-negative endocarditis in 1993, and has since emerged as a significant cause of this condition worldwide. We describe a complicated case of culture-negative endocarditis in an immune-competent male patient, which was confirmed on resected heart valves to have been caused by *Bartonella quintana* by broad-range 16S ribosomal RNA polymerase chain reaction. The objective of this report is to highlight the clinical, diagnostic and therapeutic challenges of *Bartonella* endocarditis.

S Afr Med J 2016;106(5):470-471. DOI:10.7196/SAMJ.2016.v106i5.9062

Case report

A 30-year-old man presented to his physician in October 2013 with a 6-week history of progressive dyspnoea, chest pain and non-productive cough. Of note, he had no history of congenital or rheumatic heart disease, was not known to be HIV-infected or immunocompromised from another medical condition, and was employed in the insurance industry. There was no significant history of travel or contact with domestic animals. Furthermore, he denied any intravenous drug use.

Physical examination revealed that he was pyrexial, with a pulse rate of 116 beats/minute and a blood pressure of 120/66 mmHg. He had a grade 4/6 mitral regurgitation murmur and a grade 1/4 aortic regurgitation murmur. There was no clinical evidence of micro-embolic phenomena. His spleen was just palpable under the costal margin. Chest radiography revealed biventricular cardiomegaly with upper lobe diversion, consistent with cardiac decompensation. An echocardiogram demonstrated vegetations of both the aortic and mitral valves, with incompetency across both valves. An ultrasound scan of the abdomen showed small bilateral pleural effusions, hepatosplenomegaly and a wedge-shaped zone of splenic infarction.

Laboratory investigations revealed a haemoglobin of 9.5 g/dL, leucocyte count 8.63×10^9/L, platelets 245×10^9/L, urea 10.8 mmol/L, creatinine 96 μmol/L and C-reactive protein (CRP) 42 mg/L. Urinalysis revealed >100 erythrocytes per high-power field and no casts. Four sets of blood cultures were performed, and the patient was commenced on penicillin and gentamicin for suspected native-valve endocarditis. A further three sets of blood cultures were done the following day. Rheumatoid factor was 393 IU/mL with negative antinuclear antibodies, antibodies to extractable nuclear antigen, and antibodies to cyclic citrullinated peptide. Decreased complement C3 and C4 of 0.43 g/L and 0.07 g/L, respectively, were also noted. The blood cultures remained negative, and although the patient was stable he continued to have temperature spikes while the CRP remained largely unchanged.

Serological investigations for the common causes of culture-negative endocarditis were performed (Table 1). *Brucella* polymerase chain reaction (PCR) on blood was negative. In the interim, the penicillin was substituted with teicoplanin and the gentamicin was continued. As the patient developed a degree of renal impairment, the gentamicin was discontinued and meropenem was commenced. Finally, in view

Table 1. Serological investigations for the aetiology of culture-negative endocarditis

Organism	Result
Mycoplasma pneumoniae	IgM–; IgG+
Legionella pneumophila	IgM+ (1:100); IgG+ (1:200)
Chlamydophila psittacci	IgA–; IgM–; IgG+
Chlamydia trachomatis	IgA–; IgM–; IgG+
Bartonella henselae	IgM+; IgG+ (1:512)
Bartonella quintana	IgM+; IgG+ (1:512)
Brucella spp.	IgM–; IgG+
Coxiella burnetii	IgM–; IgGp1–; IgGp2+ (1:128)

– = negative result; + = positive result; IgGp1 = phase 1 IgG antibody; IgGp2 = phase 2 IgG antibody; titres are indicated in brackets, where available.

of the serological results and negative blood cultures, doxycycline and rifampicin were also added as per international endocarditis guidelines.[1] After the addition of the latter two antibiotics, the patient's general condition improved and his temperature spikes dissipated.

The patient was taken for valve-replacement surgery approximately 4 weeks later. The resected mitral and aortic valves were sent for microbiological culture as well as broad-range 16S ribosomal RNA (rRNA) PCR. Histological examination confirmed fibrinous vegetations with associated neutrophils, lymphocytes and histiocytes. No pathogens were detected with periodic acid-Schiff or Brown-Hopps stains. Extended microbiological culture did not yield any atypical bacteria. A 16S rRNA PCR was performed and the PCR products sequenced, which confirmed the presence of *Bartonella quintana* DNA in the resected aortic heart valve. After surgery, the patient was continued on doxycycline 100 mg 12-hourly for 3 months. He remains well on subsequent follow-up examinations.

Discussion

Bartonella spp. was first discovered in 1909 by A L Barton and described as a cause of culture-negative endocarditis in 1993.[2] Bartonellae are fastidious, oxidase-negative, pleomorphic Gram-negative bacteria. Their growth is haemin-dependent; therefore, they only grow on

enriched media that contain haemin-rich rabbit or horse blood. Routine culture protocols do not detect these organisms, and prolonged culture (up to 45 days) is required.

B. henselae is the aetiological agent of cat-scratch disease, bacillary angiomatosis, meningo-encephalitis and chronic bacteraemia. The domestic cat and its fleas (*Ctenocephalides felis*) are the associated reservoir and vector for the organism, respectively. In contrast, *B. quintana* is the cause of trench fever, an infection that resulted in significant mortality during World War I and II. This organism is transmitted by the human body louse (*Pediculus humanus)* and is responsible for infections in refugees and the homeless. *B. quintana* is also associated with bacillary angiomatosis, particularly in HIV-infected individuals and cases of chronic bacteraemia. A study in South Africa (SA), conducted between 2007 and 2009, revealed a high PCR prevalence of *Bartonella* DNA in blood specimens: 22.5% (*n*=382) in HIV-infected patients, 9.5% (*n*=42) in healthy volunteers, 23.5% (*n*=98) in domestic cats, 9% (*n*=179) in domestic dogs, and 25% (*n*=124) in rodent samples.[3] Frean *et al.*[4] found that 10% (*n*=188) of patients who presented to an outpatient HIV clinic in Johannesburg had a *Bartonella* bacteraemia detected by nested PCR performed on blood.

Bartonellae are highly adapted to their mammalian hosts and can persist in the bloodstream owing to intra-erythrocytic parasitism. This bacterium has several virulence attributes, including a type IV secretion system that delivers effector molecules that subvert host cells, induction of angiogenesis by stimulating apoptosis and activation of hypoxia-inducing factor 1, adhesins and lipopolysaccharides in its outer membrane.[5]

The first published case of *Bartonella* endocarditis in SA was reported in 1996.[6] Six species have been implicated in infective endocarditis, i.e. *B. quintana, B. henselae, B. elizabethae, B. vinsonii, B. koehlerae,* and *B. alsatica.* The majority of cases (95%) are caused by *B. quintana* (75%) and *B. henselae (*25%).[6]

Risk factors for *Bartonella* endocarditis include homelessness, alcoholism, infestation with body lice (*B. quintana*), contact with cats and pre-existing valvular disease (*B. henselae*).[7]

Raoult *et al.*,[6] who described a case series of 22 patients with *Bartonella* endocarditis, reported that the clinical features of the disease were similar to those of patients with endocarditis caused by other organisms (Duke critera), except that patients were likely to be male and had undergone valvular surgery. Moreover, only 25% of their patients with confirmed *Bartonella* endocarditis had positive blood cultures. There appears to be an unknown predilection to infect the aortic valve. Although it is generally associated with native-valve endocarditis, there are reports of aggressive prosthetic valve endocarditis.[8] The echocardiograms of the majority of patients diagnosed with *B. henselae* endocarditis are consistent with endocarditis.

Serological tests often give an indication of the likely pathogen in culture-negative endocarditis. Our case was complicated by positive acute serology for both *Legionella* spp. and *Coxiella burnetii* (phase 2 serology indicates a recent infection), which also cause culture-negative endocarditis. Serological cross-reactions with *Chlamydophila* spp. and *Coxiella* spp. (Q-fever) have also been reported.[6,9] Graham *et al.*[10] reported a case of prosthetic valve endocarditis due to Q-fever, showing seropositivity to a broad range of pathogens, including *B. quintana, B. henselae, C. burnetii, Rickettsia rickettsii* and *Ehrlichia chaffeensis.* Similar to our case, this case highlights the difficulties of making a diagnosis based on serological testing in the absence of positive microbiological culture.

The raised rheumatoid factor alerted us to the possibility of a non-infective cause of endocarditis, particularly neoplastic or autoimmune. However, the negative antinuclear antibodies and histological findings did not support this diagnosis. Interestingly, Fournier *et al.*[11] identified 19 (2.5%) of 819 endocarditis cases that had non-infective causes, such as Libmann-Sacks endocarditis. This is an important consideration

in culture-negative endocarditis cases. Of note, Bojalil *et al.*[12] found that 68% (*n*=18) of their cohort with endocarditis had a positive rheumatoid factor. Other highly specific antibodies were absent. These findings are similar to those in our case.

Histological examination of a resected valve with *Bartonella* infection often reveals inflamed connective tissue with focal granulation tissue. Warthin-Starry silver staining may reveal small, dark-staining aggregates of bacteria, but these are not specific for bartonellae. Broad-range bacterial PCR-based testing of resected cardiac valves that target the 16S rRNA gene has played an important role in the diagnosis of *Bartonella* endocarditis and other causes of culture-negative endocarditis.[13] PCR on excised heart valves is considered a sensitive and reliable method.[14] False-negative results of PCR on resected valves can occur owing to the presence of PCR inhibitors, sequence variability underlying primers, or the presence of bacterial DNA in quantities less than the limit of detection of the assay. False-positives may occur where the valve is contaminated during surgical removal or transport and processing in the laboratory. It is therefore important to correlate a positive 16S rRNA PCR and sequence with the organism that has been isolated and the clinical features of the patient. In addition to the patient described in this case report, our laboratory has diagnosed several cases of *B. quintana* endocarditis and other causes of endocarditis using a broad-range 16S rRNA PCR on excised heart valves.

The optimal therapy for *Bartonella* endocarditis is unknown. Generally, the tetracyclines (doxycycline) or a beta lactam is combined with a bactericidal agent, such as gentamicin, which is added for the initial 2 - 4 weeks of therapy.[1,15] If a patient does not tolerate gentamicin, rifampicin may be used as an alternative.[15] Most guidelines advise 6 weeks of therapy, although some authors suggest extending therapy to 3 - 6 months due to the severity of the illness, low cost of the treatment and lack of treatment duration data.[16] Many patients will, however, require valve replacement.[7]

Our report highlights the importance of *Bartonella* spp. as a cause of culture-negative infectious endocarditis and the role of broad-range bacterial molecular diagnostics as a promising tool in determining the aetiology of endocarditis as well as the difficulties in laboratory diagnosis and management of this condition.

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