Culture-negative *Bartonella* endocarditis in a patient with renal failure: the value of molecular methods in diagnosis

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ABSTRACT

Members of the genus *Bartonella* are increasingly recognised as a cause of culture-negative endocarditis, particularly in those patients with underlying risk factors (e.g., homelessness and alcoholism (*B. quintana*) or valvulopathy and cat ownership (*B. henselae*). The aortic and mitral valves are most commonly involved. Here, a case is reported of culture-negative right-sided endocarditis, without any of the above risk factors, due to *Bartonella* sp. in a 69-year-old man who presented with acute renal failure. The diagnosis was made using a broad-range 16S rRNA polymerase chain reaction (PCR) technique and direct automated sequencing on a peripheral blood sample, which was subsequently confirmed serologically. A review of the literature on *Bartonella* endocarditis is also presented. Molecular laboratory methods using peripheral blood or blood cultures may be very useful in the diagnosis of causal agents in culture-negative endocarditis and add further support to the recently inclusion of molecular (PCR) diagnosis, as a major Duke's criterion, for the diagnosis of infective endocarditis.

KEY WORDS: Bartonella infections. Endocarditis. Polymerase chain reaction. Sequence analysis, RNA.

Introduction

Bartonella species, a fastidious, slow-growing Gram-negative rod, is a recognised cause of culture-negative endocarditis (CNE).[1–18] Today, the *Bartonella* genus is generally considered to consist of 16 species, which are emerging or re-emerging pathogens.[19] This genus is a member of the α_2 *Proteobacteria*[20] and is phylogenetically closely related to *Brucella* spp., *Agrobacterium* spp., *Sinorrhizobium* spp., *Rhizobium* spp. and *Ochrobactrum* spp.

Bartonella bacilliformis, the cause of Carrion's disease (a biphasic illness consisting of an acute haemolytic anaemia, Oroya fever and a chronic form, verruga peruana) was first recognised in 1909 in the Andean region of South America, where its vector, the sandfly, is endemic.[19, 20] *B. quintana*, transmitted by the body louse, was the cause of epidemic Trench fever, a debilitating but usually self-limiting illness, during the First and Second World War.

Today, it is recognised as a cause of persistent bacteraemia, particularly in alcoholic and homeless patients. *B. henselae* is the cause of cat scratch disease. Both *B. henselae* and *B. quintana* cause bacillary angiomatosis and visceral peliosis in immunocompromised patients, particularly those infected with the human immunodeficiency virus (HIV) or in transplant patients.[19,20] *B. quintana* and *B. henselae*, along with *B. vinsonii* and *B. elizabathae* are recognised causes of CNE.[1–18]

B. henselae is a zoonosis, with the domestic cat as the natural reservoir, which is spread by the scratch, bite or lick of an infected cat or by its fleas. For *B. bacilliformis* and *B. quintana*, no reservoir other than man has been demonstrated, but there are several other *Bartonella* spp. that have only been found in animals.[20]

In this study, a 69-year-old male retired shipyard welder presented in April 2000 with a history of lethargy and flu-like symptoms for several months and a recent history of haematuria. He had a history of myocardial infarction and peptic ulcer disease. There was also evidence of asbestosis and a benign monoclonal gammopathy.

Examination showed a low-grade fever, a new systolic murmur, splenomegaly and acute renal failure. There was evidence of normochromic, mormocytic anaemia, with raised inflammatory markers. Renal biopsy showed a proliferative glomerulonephritis of immune complex type with 30% crescents. The findings were suggestive of an infection-related lesion. On echocardiography, there was tricuspid regurgitation and a large (2.2 cm) vegetation on the tricuspid valve, confirming the diagnosis of right-sided infective endocarditis with secondary acute glomerulonephritis. Renal function deteriorated and the patient required haemodialysis.

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Nine sets of blood cultures were taken. All were negative apart from a significant coagulase-negative staphylococci infection acquired via the dialysis access line. The patient had received a course of ciprofloxacin before admission and a short course of teicoplanin, cefotaxime and clarithromycin, prior to most of these cultures.

Serology for *Coxiella burnetii*, *Chlamydia psittaci*, *Brucella* and *Legionella* spp. were negative. In an effort to define a pathogen, a broad-range 16S rRNA polymerase chain reaction (PCR) technique was undertaken on a BacTAlert blood culture and on a sample of peripheral blood.

Materials and methods

Total genomic bacterial DNA was extracted from blood culture material as previously described.[21] For each batch of extractions, a negative extraction control (all reagents but minus blood or blood culture) and a positive extraction control (a positive blood culture) were performed. Fresh peripheral blood (approximately 500 µL) was collected in a sterile plastic vial (2 mL) containing 0.5% (w/v) EDTA (100 µL).

Initially, each blood–EDTA sample was washed with 10 mmol/L Tris–HCl (1 mL; pH 8.0) and centrifuged (13,000 x*g*, 5 min) to remove haemoglobin, which is a strong inhibitor of PCR amplification. The resulting pellet was resuspended in 10 mmol/L Tris–HCl (200µl) and incubated with 20µL lysozyme (10 mg/mL in Tris–HCl) at 37°C for 30 min. Genomic and human DNA was extracted subsequently as per the manufacturer's instructions, using the QIAamp blood kit (Qiagen, UK). All extracted DNA samples were stored at –20°C prior to use.

The PCR technique was performed on extracted DNA from blood culture and peripheral blood–EDTA employing broad-range 16S rRNA primers. Two approaches were used: i) a non-nested PCR amplification employing the primer set P11P/P13P;[21] and ii) a nested PCR amplification employing the primer sets (first round: PSL/PSR, second round: P11P/P13P).[21] Positive amplicons were sequenced on the ALF II Express automated sequencer (Amersham Pharmacia, England) and the resulting sequences were compared, using the Basic Local Alignment Software Tool (BLAST) (www.blast.genome.ac.jp), with sequences deposited in GenBank (AF409997).

Results

Following the single-round PCR amplification, no amplicon was detected in blood culture material and only a weak positive product was obtained from the blood–EDTA specimen, which proved difficult to sequence directly. In order to improve the sensitivity of the assay, a nested PCR approach was adopted, which yielded an amplification product that could be sequenced directly, yielding a sequence (GenBank accession number AF409997) comparable to that of *Bartonella* spp. (100% homology with 153 bases called). The molecular finding was corroborated by serological investigation using indirect immunofluorescence, which revealed an antibody titre of 1 in 1600 for *B. henselae houston, B. henselae marseille, B. quintana* and *B. clarridgeiae*. This titre has been shown to have a positive predictive value for endocarditis of 0.884.[22]

The patient was treated for six weeks with intravenous imipenem (500 mg twice daily and 250 mg post-haemodialysis), intravenous netilmicin (150 mg once daily as per levels) and ciprofloxacin (750 mg orally twice daily). Thereafter, he continued on ciprofloxacin and rifampicin (300 mg orally twice daily). Due to nausea, ciprofloxacin was later changed to coamoxiclav (375 mg orally three times a day) and rifampicin. Treatment was continued for six months. Renal function improved and haemodialysis was discontinued. The patient remained well 15 months after discharge; however, significant renal impairment persisted (creatinine: 300 µmol/L, creatinine clearance: 33 mL/min) and he may eventually return to dialysis.

Discussion

In a review of 22 cases of *Bartonella* endocarditis by Raoult *et al*.,[1] 17/22 (77%) patients were male and the median age was 47 years. The aortic valve alone was involved in 17/22 (77%), 20/22 (91%) required valve surgery and death related to endocarditis occurred in 6/19 (32%). In a review of 24 other cases, including the one reported here (Table 1), similar results were seen (22/24 [92%] males, median age: 42.5 years). The aortic valve alone was involved in 12/24 (50%), 20/24 (83%) required surgery (two patients were unfit and died before surgery) and 5/24 (21%) died. *Bartonella* endocarditis of the tricuspid valve alone has been described before[13] and, as in this previous case, the patient reported here was cured without surgery, although he required a prolonged course of antibiotics.

Based on the limited knowledge of antimicrobial sensitivity for *Bartonella* spp.,[23–25] penicillin, third-generation cephalosporins, imipenem, aminoglycosides, fluoroquinolones, rifampicin, doxycycline and erythromycin have shown good *in vitro* activity. In one study of *B. henselae*, aminoglycosides were the only antibiotics that demonstrated bactericidal activity.[25] Clinical experience[1–18] (Table 1) has shown that antibiotics capable of intracellular penetration (e.g., fluoroquinolones, tetracyclines, macrolides and rifampicin) are also associated with a good outcome. The patient reported here responded well to a combination of a carbapenem, aminoglycoside and an oral fluoroquinolone, followed by a combination of an oral fluoroquinolone and rifampicin, and then coamoxiclav and rifampicin.

Culture-negative endocarditis is a significant clinical problem, accounting for 5–24% of cases of endocarditis.[26] In a study of endocarditis at St. Thomas' Hospital, London, between 1985 and 1996, 10% of 221 cases were culture-negative.[11] However, when clinical situations involving prior antibiotic use were excluded, only some 5% of cases of endocarditis prove to be genuinely culture-negative.[11,27]

Molecular methods can be very useful in the investigation and diagnosis of culture-negative endocarditis, and Millar *et al*.[27] have argued for their inclusion as a new Duke's criterion. In this study, PCR amplification of universal loci (16S rRNA for bacteria and 18S, 28S and 5.8S rRNA for fungi), and additional sequence specific gene loci (e.g., *femB* for *Staphylococcus aureus*, *mecA* for methicillin-resistance and the 16S–23S rRNA intergenic spacer region for *Bartonella* spp., which gives an amplified product of varying length

Table 1. Overview of reported cases of *Bartonella* endocarditis.

Patient (Ref)	Sex	Age(years)	Previous valve disease/ other risk factors	Valve involved (emboli)	Organism Laboratory result
1 (2)	M	50	HIV + Cat owner	Mitral & aortic	*B. quintana* Bl cul +
2 (3)	M	59	Alcoholic	Aortic & tricuspid	*B. henselae* PCR (V-F) +
3 (4)	M	31	(infected teeth, *Strongyloides* in stool)	Aortic	*B. elizabethae* B.cul +
4 (5)	M	46	C_2H_5OH Poor dentition	Aortic (Brachial artery embolus)	*B.quintana* PCR (V) + Serology +
5 (6)	M	39	Homeless C_2H_5OH Poor dentition	Aortic	*B. quintana* B cul + PCR (V-F) +
6 (7)	M	41	Bicuspid AV, mild AS Cat owner	Aortic	*B.henselae* Serology + PCR (V) + PCR (LN) +
7 (8)	M	47	Heart murmur Homeless C_2H_5OH Dog contact	Mitral	*B. quintana* Bl cul + Serology + PCR (V-F) +
8 (8)	M	41	Homeless C2H5OH Cat, dog, goat contact	Aortic (peripheral emboli)	*B. quintana* Bl cul (CC) + Serology + PCR (V-F) +
9 (8)	M	43	Homeless C_2H_5OH	Aortic & mitral	*B. quintana* Bl cul (CC) + Serology + PCR (V-F) +
10 (9)	M	22	Bicuspid AV, VSD Farm worker, Owns Cat	Aortic & tricuspid	*B. henselae* serotype Marseille Serology + B cul (CC)+ PCR (V) +
11 (10)	M	34	? Hx valvular disease* (Algeria)	Aortic (?embolus)	*Bartonella* sp. Serology + & ?
12 (11)	M	55	Homeless C_2H_5OH, poor dentition, dog contact	Mitral & aortic	*B. quintana* Serology + PCR (V) +
13 (11)	M	41	Bicuspid AV, RA, iritis, owns cat.	Aortic (branch retinal art embolus)	*B. henselae* Serology + PCR (V) +
14 (12)	M	38	Homeless Poor dentition Schizophr	Aortic & mitral Probable IE	*Bartonella* sp. Serology +
15 (13)	M	65	C_2H_5OH	Tricuspid	*Bartonella* sp.$^{\delta}$ Serology +
16 (14)	F	4	Bicuspid AV VSD, Hx of repaired coarctation Cat owner	Aortic	*B. henselae* PCR (V-?F) + Serology +
17 (15)	M	50	C_2H_5OH Poor dentition	Mitral & aortic	*B. quintana* Bld cul + Serology +
18 (16)	M	35	Bicuspid AV Owns cat & dog Contact with pig, cow & cockerel in Portugal	Aortic	*B. vinsonii* subsp. *berkhoffii* PCR (V) + Serology +
19 (17)	F	13	Rheumatic heart disease (Senegal)	Mitral & Aortic Interventr septum	*B.quintana* PCR (V) + Serology +
20 (18)	M	45	Homeless C2H5OH IVDA	Aortic	*B. quintana* V cul + PCR (V) + Serology +
21 (18)	M	59	Hx: Mixed aortic valve disease	Aortic	*B. quintana* V cul + PCR (V) + Serology +
22 (18)	M	69		Mitral	*Bartonella* sp. Serology +
23 (18)	M	42	C_2H_5OH Psychiatric Hx	Aortic	*Bartonella* sp. Serology +

AS, Aortic stenosis; Bl cul, Blood culture; Bl cul (CC), Blood culture (cell line culture); PCR (V-F), polymerase chain reaction of valve – formalin fixed; PCR (V), PCR of valve; PCR (LN), PCR of lymph node; Hx., History of...; V, valve; RA, rheumatoid arthritis; Art, artery; A. root replace, aortic root replacement; *other risk factors (e.g., C_2H_5OH, homelessness not available); Schizophr, schizophrenia; IV, intravenous; PO, oral; δ, Presumed *B. quintana*. Interventr septum, interventricular septum; IVDA, intravenous drug abuser.
Valve culture may be from agar plate inoculation or cell-line inoculation and culture.
PCR-positive valve may be from fresh valve or formalin fixed tissue specimens.

depending on which species is present) were used. A high level of agreement was found between conventional blood culture and molecular detection in 11 definite cases of endocarditis, while 41 culture-negative cases rejected by Duke's criteria were also negative by molecular methods.

Bartonella spp. are recognised increasingly as a cause of culture-negative endocarditis and were estimated by Raoult *et al*. to be responsible for 3% of cases.[1] It is a fastidious organism and is difficult to grow on blood agar. Serology is not always positive and therefore, if positive, molecular

methods are very useful in establishing a diagnosis.

In the case reported here, there were no obvious risk factors for *Bartonella* infection. Broad-range PCR molecular methods established the diagnosis (with subsequent serological confirmation), resulting in a significant change in management and a successful outcome for the patient. In addition to its microbiological interest, the case shows the value of renal biopsy in the clinical management of unexplained acute renal failure. Owing to the patient's co-existing gammopathy, the preferred initial diagnosis was renal amyloidosis; however, the biopsy finding of acute glomerulonephritis led to an intense clinical search for endocarditis, which might otherwise have been missed.

In conclusion, the use of broad-range PCR techniques and direct automated sequencing of resulting amplicons may be useful in the diagnosis of culture-negative endocarditis, as demonstrated in this case.

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References

1 Raoult D, Fournier PE, Drancourt M *et al*. Diagnosis of 22 new cases of *Bartonella* endocarditis. *Ann Intern Med* 1996; **125**: 646–52.

2 Spach DH, Callis, KP, Paauw DS *et al*. Endocarditis caused by *Rochalimaea quintana* in a patient infected with the human immunodeficiency virus. *J Clin Microbiol* 1993; **31**: 692–4.

3 Hadfield TL, Warren R, Kass M, Brun E, Levy C. Endocarditis caused by *Rochalimaea henselae*. *Hum Pathol* 1993; **24**: 1140–1.

4 Daly JS, Worthington MG, Brenner DJ *et al*. Rochalimaea elizabathae sp. nov. isolated from a patient with endocarditis. *J Clin Microbiol* 1993; **31**: 872–1.

5 Jalava J, Kotilainen P, Nikkari S *et al*. Use of the polymerase chain reaction and DNA sequencing for detection of *Bartonella quintana* in the aortic valve of a patient with culture-negative infective endocarditis. *Clin Infect Dis* 1995; **21**: 91–6.

6 Spach DH, Kanter AS, Daniels NA *et al*. *Bartonella (Rochalimaea)* species as a cause of apparent 'culture negative' endocarditis. *Clin Infect Dis* 1995; **20**: 1044–7.

7 Holmes AH, Greenough TC, Baladt GJ *et al*. *Bartonella henselae* endocarditis in an immunocompetent adult. *Clin Infect Dis* 1995; **21**: 1004–7.

8 Drancourt M, Mainardi JL, Brouqui P *et al*. *Bartonella (Rochalimaea) quintana* endocarditis in three homeless men. *N Engl J Med* 1995; **332**: 419–23.

9 Drancourt M, Birtles R, Chaumentin G, Vandenesch F, Etienne J, Raoult D. New serotype of *Bartonella henselae* in endocarditis and cat scratch disease. *Lancet* 1996; **347**: 441–3.

10 Mainardi J, Drancourt M, Roland J *et al*. A new case of *Bartonella (Rochalimaea) quintana* endocarditis. *Clin Microbiol Infect* 1996; **1**: 275–6.

11 Breathnach AS, Hoare JM, Eykyn SJ. Culture-negative endocarditis: contribution of Bartonella infections. *Heart* 1997; **77**: 474–6.

12 Case Records of the Massachusetts General Hospital, Weekly Clinicopathological Exercises. Scully RE, Mark EJ, McNeely WF, Ebeling SH eds. Case 2, 1997. *N Engl J Med* 1997; **336**: 205–10.

13 Bruneel F, D'estanque J, Fournier PE *et al*. Isolated right-sided *Bartonella quintana* endocarditis in an immunocompetent adult. *Scand J Infect Dis* 1998; **30**: 424–5.

14 Baorto E, Payne RM, Slater LN *et al*. Culture-negative endocarditis caused by *Bartonella henselae*. *J Pediatr* 1998; **132**: 1051–4.

15 Guyot A, Bakhai A, Fry N, Merritt J, Malnick H, Harrison T. Culture-positive *Bartonella quintana* endocarditis. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 145–7.

16 Roux V, Eykyn SJ, Wyllie S, Raoult D. *Bartonella vinsonii* subsp. *berkhoffii* as an agent of afebrile blood culture-negative endocarditis in a human. *J Clin Microbiol* 2000; **38**: 1698–700.

17 Barbe KP, Jaeggi E, Ninet B *et al*. *Bartonella quintana* endocarditis in a child. *N Engl J Med* 2000; **342**: 1841–2.

18 James EA, Hill J, Uppal R, Prentice MB. *Bartonella* infection: a significant cause of native valve endocarditis necessitating surgical management. *J Thorac Cardiovasc Surg* 2000; **119**: 171–2.

19 Anderson BE, Neuman MA. *Bartonella* spp. as emerging human pathogens. *Clin Microbiol Rev* 1997; **10**: 203–19.

20 Maurin M, Birtles R, Raoult D. Current knowledge of *Bartonella* species. *Eur J Clin Microbiol Infect Dis* 1997; **16**: 487–506.

21 Xu J, Millar BC, Moore JE *et al*. Employment of broad-range 16S rRNA PCR to detect aetiological agents of infection from clinical specimens in patients with acute meningitis – rapid separation of 16S rRNA PCR amplicons without the need for cloning. *J Appl Microbiol* 2003; **94**: 197–206.

22 Raoult D, Tissot Dupont H, Enea-Mutillod M. Positive predictive value of *Rochalimea henselae* antibodies in the diagnosis of cat scratch disease. *Clin Infect Dis* 1994; **19**: 335.

23 Maurin M, Raoult D. Antimicrobial susceptibility of *Rochalimaea quintana, Rochalimaea vinsonii* and the newly recognised *Rochalimaea henselae*. *J Antimicrob Chemother* 1993; 32: 587–94.

24 Maurin M, Gasquet S, Ducco C, Raoult D. MICs of 28 antibiotic compounds for 14 *Bartonella* (formerly *Rochalimaea*) isolates. *Antimicrob Agents Chemother* 1995; **39**: 2387–91.

25 Musso D, Drancourt M, Raoult D. Lack of bactericidal effect of antibiotics except aminoglycosides on *Bartonella (Rochalimaea) henselae*. *J Antimicrob Chemother* 1995; **36**: 101–8.

26 Millar BC, Altwegg M, Raoult D, Moore JE. Culture-negative endocarditis – causes, diagnosis and treatment. *Rev Med Microbiol* 2000; **11**: 59–75.

27 Millar B, Moore J, Mallon P *et al*. Molecular diagnosis of infective endocarditis – a new Duke's criterion. *Scand J Infect Dis* 2001; **33**: 673–80.