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### Endocarditis in a Dutch patient caused by *Bartonella quintana*

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Spach et al, in 1993, were the first to describe the isolation of *Bartonella quintana* from an HIV-infected patient with endocarditis [1]. Since then, several groups have reported cases of endocarditis in immunocompetent patients caused by *Bartonella quintana* (in the majority of cases), *Bartonella henselae*, or *Bartonella elizabethae* (one case) [2–11]. Diagnosis was based on culture, serologic studies or polymerase chain reaction (PCR) detection. Here we report the detection and identification of *Bartonella quintana* in the aortic valve of a Dutch patient with culture-negative endocarditis.

A 74-year-old man (not homeless or otherwise neglected) was admitted to hospital because of general malaise, weight loss and leukocytosis. A year earlier, he had been bitten by an insect on the ankle, which remained swollen for several weeks. The patient did not own domestic animals. No further abnormalities, especially no heart murmurs, were found on physical examination. In the days following admission, his temperature rose to 39°C and his condition deteriorated. Laboratory investigations showed an erythrocyte sedimentation rate (ESR) of 70 mm/h, hemoglobin 5.7 mmol/L, thrombocytes  $179 \times 10^9/L$  and leukocytes  $40.2 \times 10^9/L$ . Liver function tests and ECG were normal. Bacteriologic cultures of urine, bone marrow and multiple blood samples were negative. Bone marrow

examination indicated myeloproliferative activity, without the typical picture of chronic myeloid leukemia. Cytogenetic examination did not reveal a Philadelphia translocation. Serologic tests for *Treponema pallidum* and *Coxiella burnetii* were negative. However, a *Borrelia burgdorferi*-specific immunofluorescence assay (IFA) revealed an antibody titer of 1:1280. Because the general condition of the patient gradually worsened, and because of the positive *Borrelia* serology, empirical intravenous treatment with amoxicillin-clavulanic acid was started. Following this treatment, the condition of the patient improved and the leukocyte count normalized. Antibiotic treatment of the patient was continued using oral amoxicillin 500 mg three times daily for 4 weeks. ESR and leukocyte count reached their lowest levels of 36 mm/h and  $9.7 \times 10^9/L$ , respectively, at 6 months after admission; leukocyte differentiation at that point was normal. Seven months after the first hospitalization, the patient was readmitted to the hospital with complaints of dyspnea and tiredness. At this stage, a heart murmur was heard. A chest X-ray showed cardiomegaly, a pleural effusion and interstitial edema. The leukocyte count had risen to  $30.4 \times 10^9/L$ . Ultrasound examination of the heart showed mitral valve insufficiency and severe aortic valve insufficiency with valvular vegetations, indicating endocarditis. Despite intravenous treatment with ampicillin and gentamicin, the patient developed fever, renal insufficiency, and increasing cardiac failure. He underwent valve replacement and hemodialysis. Microscopic examination of the aortic valve revealed the presence of sclerotic vegetations, but there was no indication of active endocarditis. The patient died 3 weeks after surgery, from septicemia and pulmonary hemorrhage.

Within a 9-month period in 1995, five serum samples were taken from the patient. All sera were tested for the presence of IgG and IgM antibodies against *Bartonella*, in an enzyme-linked immunoassay (EIA) using *Bartonella henselae* (ATCC 49882) and *Bartonella quintana* (90-268) as antigens, and in an IFA using *Bartonella henselae* (ATCC 49882) as antigen (Table 1) [12]. Although the *Borrelia*-specific IFA

**Table 1** Serologic results of sera from a patient with *Bartonella quintana* endocarditis

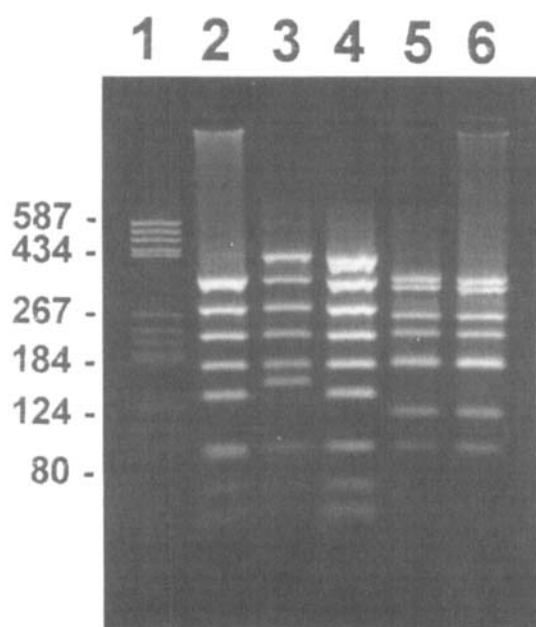
Date	EIA IgG <i>B. henselae</i>	EIA IgM <i>B. henselae</i>	EIA IgG <i>B. quintana</i>	EIA IgM <i>B. quintana</i>	IFA IgG <i>B. henselae</i>	IFA IgM <i>B. henselae</i>
22/03/95	73 600	200	19 000	288	NT	32–64
30/03/95	61 700	200	17 100	273	NT	32–64
03/11/95	80 300	218	17 800	370	NT	>=128
13/11/95	82 500	200	16 300	323	NT	>=128
04/12/95	103 400	200	19 500	206	>=256	32

NT, titer could not be determined because of high background.

**Table 2** Serologic results of sera from the relatives of the patient

Number	Age (years)	EIA IgG	EIA IgM	EIA IgG	EIA IgM	IFA IgG	IFA IgM
		<i>B. henselae</i>	<i>B. henselae</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. henselae</i>	<i>B. henselae</i>
1	77	947	<200	370	<200	32	<8
2	67	<200	<200	<200	<200	<16	<8
3	69	365	<200	449	<200	<16	<8
4	72	1350	<200	562	<200	32	<8
5	32	337	<200	304	<200	16	<8
6	76	1600	<200	869	<200	32	<8
7	69	<200	<200	<200	<200	<16	<8

initially indicated the presence of high *Borrelia burgdorferi*-specific antibody titers, Western blotting analysis and *Borrelia burgdorferi*-specific EIA did not reveal *Borrelia burgdorferi*-specific IgG or IgM antibodies in any of the patient's serum samples (data not shown). Eleven months after the onset of disease, sera were taken from seven relatives who lived in close contact with the patient. The sera were tested for the presence of *Bartonella henselae* and *Bartonella quintana* antibodies (Table 2).



**Figure 1** Detection and identification of *Bartonella* DNA in the aortic valve from a Dutch patient with endocarditis by PCR-RFLP analysis of the 16S-23S spacer region using broad-host-range primers, and restriction endonuclease *AluI*. Lane 1, molecular weight marker (marker V, Boehringer Mannheim, Germany); lanes 2-4, *Bartonella henselae* strains with distinct *AluI* RFLP patterns; lane 2, ATCC 49882; lane 3, ATCC 49793; lane 4, 91-148; lane 5, *Bartonella quintana* strain 92-263; lane 6, aortic valve vegetation from patient with endocarditis.

DNA was extracted from the aortic valve vegetation as described earlier [13], and the DNA extracts were analyzed by three different DNA methods. PCR with the *Bartonella*-specific primers p24E and p12B described by Relman et al yielded a PCR fragment of the expected size of approximately 300 bp [14]. This PCR fragment strongly reacted with a 5'-biotinylated, *Bartonella quintana*-specific oligonucleotide probe (5'-ATTAAGTTGGGCACTCTAGGGG-3') in a Southern blot hybridization. *AluI* polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S-23S rRNA spacer region according to Matar et al revealed an RFLP pattern identical to that of *Bartonella quintana* strain 92-263, described by Welch et al [15-17] (Figure 1). PCR with the broad-host-range 16S rRNA gene primers 16S8FOR (5'-AGAGTTTGATC(AC)TGG(TC)TCAG-3') and 16S575REV (5'-CTTTACG CCA(AG)T(AG)A(AT)TCCG-3') yielded a high amount of a PCR fragment of approximately 525 bp. DNA sequence analysis of this PCR fragment revealed 100% identity with two 16S rRNA gene sequences of *Bartonella quintana* present in the EMBL nucleotide sequences database (accession numbers U28268 and M73228). Blood cell DNA extracts from the patient's relatives were tested for the presence of *Bartonella* DNA in a PCR with the *Bartonella*-specific primers described above. None of those DNA extracts contained detectable *Bartonella* DNA. Blood cells from the patient were not available for DNA analysis.

We propose *Bartonella quintana* as the cause of the patient's subacute endocarditis on the basis of a positive *Bartonella* PCR, hybridization with a *Bartonella quintana*-specific DNA probe, 16S-23S spacer RFLP analysis, and partial 16S rRNA gene sequence analysis on DNA extracts from the aortic valve. Additionally, we found high IgG and IgM titers against both *Bartonella henselae* and *Bartonella quintana*. The positive *B. henselae* serology was probably due to cross-reactivity between *Bartonella quintana* and *Bartonella henselae* [11,18]. The EIA IgG and IgM antibody titers found in the patient were elevated during the 9-month period of the disease, and

IgM antibodies could be detected by EIA over a period of nearly 8 months. This case has three remarkable aspects. First, it shows again that the diagnosis of endocarditis can be missed in the evaluation of fever of unknown origin when physical examination reveals no abnormalities and repeated blood cultures are negative. Second, (false-)positive *Borrelia* serology, together with some aspects of the history such as an insect bite, led to a wrong working diagnosis during the first episode of the disease. Third, leukocytosis and myeloproliferative reaction of the bone marrow, although rather non-specific features, were pronounced, and this has not been described earlier in patients with endocarditis due to *Bartonella*; leukocyte counts reported until now in those patients have ranged from  $1.7 \times 10^9/L$  to  $13.0 \times 10^9/L$  [1,3,5–10].

The route of transmission of *Bartonella quintana* is not known. The epidemiology of trench fever implies inter-human transmission of *Bartonella quintana* via the body louse. The patient described in this report kept good care of his personal hygiene, clothes and housing. No evidence of infestation by body lice was found. He did not use alcohol or drugs, and was not diabetic, and there was no reason to perform HIV serology. He had frequent contact with his relatives, and he lived with his sister for some months after his first stay in the hospital. The fact that none of the tested relatives had IgM antibodies to *Bartonella* species indicates that transmission of *Bartonella quintana* from or to those persons is not very likely. The patient's vegetable garden, from which he had occasionally removed dead rats (mammals that may serve as a reservoir), was located in a semirural area. Alternatively, he may have acquired his infection from the insect bite that he sustained earlier.

This is the first report of *Bartonella* endocarditis in the Netherlands. Worldwide, about 34 cases of *Bartonella* endocarditis have been reported [1–11]. Twenty-nine of these 34 patients (85%) were male, 17 of them (50%) had a history of alcohol abuse, and 13 (38%) were homeless. They were between 22 and 81 years of age, one of them was HIV seropositive, and nine (26%) reported contact with animals (cats, dogs, goat). The course of infection included fever in 29 cases (85%), and valve resection was necessary in 30 patients (88%), despite intravenous antibiotic treatment. Raoult et al recently showed that alcoholism and homelessness without previous valvular heart disease are risk factors for *Bartonella quintana* endocarditis, but not for endocarditis due to other *Bartonella* species [11].

The incidence of endocarditis due to *Bartonella* may be much higher than we know. Many cases may be unrecognized due to the slow growth of *Bartonella* species. The implementation of recently developed molecular techniques may lead to an increase in the

number of reports on endocarditis due to *Bartonella* species over the next few years.

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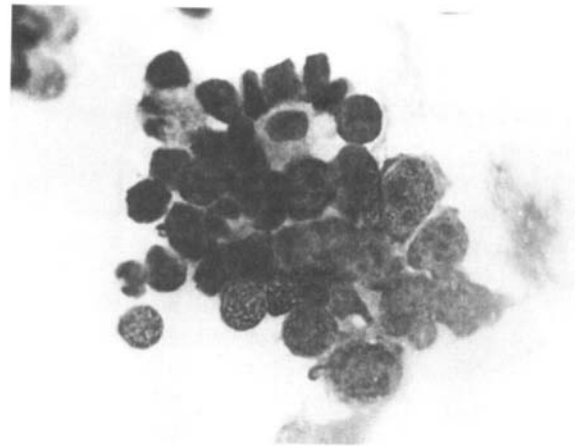
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#### Primary leptomeningeal lymphoma simulating chronic meningitis

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A 33-year-old male presented with increasing headache, vomiting and weakness, and gave a history of weakness for 2 months and an attack of hemiparesis and speech difficulty 1 month before, which improved spontaneously in half an hour. Physical examination was unremarkable on admission, except that two seizures were observed, separated by 5 min, which stopped spontaneously in 10–15 s. A few days later, his headache increased, and somnolence, visual loss, depressed deep tendon reflexes, loss of the plantar response, stiff neck and Kernig's and Brudzinski's signs developed. He



**Figure 1** Malignant cells in cerebrospinal fluid.

had a moderate fever but only on the few days on which he had herpetic lesions on his lips, nose and face.

The cerebrospinal fluid (CSF) examinations showed a clear CSF, with at first normal, but later increased, pressure, a pleocytosis of 100–430 cells/mm<sup>3</sup> with lymphocytic predominance (lymphocytes 65–100%, mostly atypical; neutrophils 0–35%), hypoglycorrhachia (the CSF/blood glucose ratio being 1/3:1/14), increased protein levels (Pandy positive, protein levels 48–61 mg/dL) and lactic dehydrogenase levels of 212–790 mg/dL. The microbiological and serologic evaluations of CSF and blood were negative (Gram, Ziehl–Neelsen, Giemsa, India-ink stainings; cultures; tests for brucellosis, syphilis, toxoplasmosis, tuberculosis, cytomegalovirus, Epstein–Barr virus, herpes simplex virus or human immunodeficiency virus infections). The cytopathologic examination of CSF by cytocentrifugation, drying the preparations at room temperature in air and May–Grünwald–Giemsa and periodic acid–Schiff (PAS) stainings showed PAS-positive malignant cells of lymphoblastic type (Figure 1). The complete blood count, erythrocyte sedimentation rate, urine examination, blood chemistry and serum protein electrophoresis were normal. The tuberculin test was negative and bone marrow aspiration was normal. Chest and paranasal sinus radiographs and thoracic and abdominal computed tomography (CT) scans were normal. Cranial CT and magnetic resonance imaging of the brain revealed ventricular enlargement, but no space-occupying lesion. The patient was accepted as a case of primary leptomeningeal lymphoma (PLML), and responded well to intrathecal chemotherapy.

In large series, primary central nervous system lymphoma (PCNSL) represents 0.5–1.5% of all primary brain tumors and 0.5–2% of all non-Hodgkin's lymph-