Osteomyelitis Caused by *Bartonella henselae* Genotype I in an Immunocompetent Adult Woman

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We report the first description of osteomyelitis due to *Bartonella henselae* genotype I in an immunocompetent middle-aged woman. The diagnosis was established by serology, histopathology, and PCR analysis of osseous and lymph node tissues. The mycobacteria growth indicator tube inoculated with the lymph node aspirate was used for PCR analysis.

CASE REPORT

A 62-year-old woman presented with a 2-week history of cervical column tumefaction. She complained of severe pain in the cervical column with radiation of the pain and paresthesia in both arms. She had intermittent fevers and night sweats. Her medical history included an epithelioma of the psoas treated by radiotherapy at age 49 and an endometrial carcinoma treated by surgery and radiotherapy at age 50. She was initially taking no medication. She lived in Belgium and owned about 20 cats.

Clinical examination revealed a left cervical tumefaction. The remainder of the examination, including the neurological one, revealed no abnormalities. Hemoglobin and leukocyte levels were normal. C-reactive protein level was elevated, at 12.3 mg/liter. Tumor markers were normal. HIV serology was negative. Ultrasound of the cervical tumefaction suggested a buildup of necrotic lymphadenopathies. A computed tomography scan showed the presence of a paravertebral mass with involvement of the C5 and C6 cervical vertebrae, suggestive of metastatic infiltration. In view of her past medical history, the initial diagnosis was a recurrence of her endometrial carcinoma. A needle aspiration of the cervical tumefaction was undertaken. Histological examination showed necrotizing granulomatous inflammation indicative of cat scratch disease (CSD). Serological testing done by indirect immunofluorescence on *Bartonella henselae* slides commercialized by Focus Technologies (Cypress, California) was positive for immunoglobulin G (IgG), with a titer at 256. Magnetic resonance imaging (MRI) performed a few days later showed spondylitis with diskitis of the C5 and C6 vertebrae, phlegmona at the same level, and left cervical lymphadenopathies suggestive of tuberculosis. A Mantoux test was negative. Chest radiography was normal. Mycobacterial cultures from the feces and the gastric liquid were made. The patient was discharged with a suspicion of *Mycobacterium tuberculosis* infection. The C-reactive

protein level was at 80 mg/liter, and the white blood cell count was 14.8×10^9 /liter with a neutrophil count of 12.8×10^9 /liter.

Diagnostic and therapeutic C5 and C6 discectomy and arthrodesis by autograft were performed 2 weeks later. Several milliliters of pus as well as an osseous fragment of C6 were collected. Bacterial and mycobacterial cultures failed to grow any organisms. Histological examination showed granulomatous lesions indicative of CSD. PCR confirmed the presence of *B. henselae* DNA. Extraction was done by a commercially available DNA purification kit (Dneasy tissue kit; Qiagen, Westburg, The Netherlands). PCR with the degenerate primers CAT1 and CAT2, which enable amplification of a 414-bp fragment of the heat shock gene (*htrA*) of *B. henselae* and *Bartonella quintana*, was performed according to the method of Anderson et al. (1). The identity of the PCR product was checked by a Southern blot hybridization assay with probe RH1, which enables the differentiation of *B. henselae* and *B. quintana* (1). A subsequent type-specific PCR by the method of Bergmans et al. (5) revealed the presence of genotype I *B. henselae*. The patient received intravenous ofloxacin and clindamycin during 3 weeks. She had a favorable clinical response. Her white blood cell count returned to normal 2 weeks after surgery, and her C-reactive protein level returned to normal after 6 weeks, whereas IgG antibodies against *B. henselae* disappeared after 1 week. She was discharged with prescriptions for a 2-month oral therapy of ofloxacin (400 mg twice daily) and a 6-month therapy of clarithromycin (500 mg twice daily).

However, 2 months after surgery, a new tumefaction appeared in the left cervical region. Serological testing showed detectable IgG and IgM antibodies against *B. henselae*, with titers, respectively, at 256 and 64. The collection was localized in the same anatomical region as the previous one, and MRI showed no communication with the C5–6 arthrodesis. The collection was drained surgically. Histological examination showed remains of lymph node tissue as well as granulomatous lesions indicative of CSD. Bacteriological and mycobacteriological stains and cultures remained negative. The inoculated mycobacteria growth indicator tube (MGIT) was used for *B. henselae* PCR analysis, which was positive. Type-specific PCR again revealed the presence of *B. henselae* genotype I.

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Control MRI 9 months after surgery showed a complete consolidation of the cervical autograft with no more signs of infection.

Discussion. CSD usually presents as a self-limited lymphadenopathy following a cat scratch. Atypical presentations, such as ocular involvement, encephalopathy, hepatosplenic infection, endocarditis, or osteomyelitis, are seen in up to 14% of the cases (9, 16). Osteomyelitis associated with CSD occurs mainly in children (13, 18). So far, there have only been four reports of CSD osteomyelitis in immunocompetent adults (12, 14, 21, 22). It can affect any bones, including the vertebrae. Lymphadenopathy is usually found in association with the bone lesions, either concomitantly or after a period of several weeks. The spread of the bacteria from the inoculation site to the bone has been proposed to be hematogenous, lymphatic, or contiguous. We think that in this case, the spreading mode was contiguous from an involved cervical lymph node adjacent to the vertebral body. Most patients recover from CSD osteomyelitis and it is not clear whether antibiotics contribute to the recovery (13, 18). In our case, however, surgery had to be undertaken. The second episode of lymphadenopathy might be due to persistent cervical bacteria despite oral antibiotic therapy. It might also result from a second inoculation of *B. henselae*, as our patient was indeed frequently scratched by her many cats.

Most of the CSD osteomyelitis cases were reported before serological tests for *B. henselae* became available. As culture and isolation of *B. henselae* are very difficult, diagnosis was based on the presence of chronic peripheral lymphadenopathy, a history of cat exposure, a positive cat scratch skin test, and a histological examination of lymph nodes or bone tissue. Today, the skin test is no longer utilized, given the potential risk of transmitting infectious agents (2). Histopathology of the osseous and lymph node material usually reveals suppurative granulomas with central necrosis containing disintegrating neutrophils surrounded by palisading epithelioid cells and lymphocytes. In some cases, granulomas may contain central homogenous acellular material resembling the caseous necrosis encountered in *M. tuberculosis* infection. This picture may render the differential diagnosis with tuberculosis difficult. This is particularly problematic when clinical and radiological presentations are compatible with tuberculosis, as in our case. *B. henselae* serology and PCR are very useful current tools to confirm CSD osteomyelitis. Serology was positive in most of the CSD osteomyelitis cases (13, 14, 18, 21). Serology in our case was done by indirect immunofluorescence, and the cutoff values for positive serology were at a dilution of 1/128 for IgG and of 1/64 for IgM (7). Positive PCR has been reported in only two lymph nodes and in six osseous tissues of CSD osteomyelitis (6, 10, 13, 14, 17, 18, 21). In our case, we tested both the osseous and the lymph node materials. No pus aspiration from the lymph node was available for PCR. We therefore extracted the MGIT liquid medium that had been processed for mycobacterial culture. To our surprise, *B. henselae* PCR was positive. Since MGIT-processed media are usually kept for 42 days, such a procedure might be helpful to diagnose CSD retrospectively, when no material is available anymore. This is particularly

important, given the often difficult differential diagnosis between CSD and tuberculosis and the key position of *B. henselae* PCR in the final diagnosis of these cases. *M. tuberculosis* culture is indeed positive in only 43% of lymph node tuberculosis patients (11), whereas *Bartonella* PCR is positive in up to 98% of CSD patients when fresh lymph nodes are analyzed (4; S. Woestyn, N. Olivé, G. Bigaignon, and M. Delmée, unpublished data).

This is the first description of CSD osteomyelitis due to *B. henselae* genotype I. Two genotypes based on 16S rRNA sequences have been described in uncomplicated CSD (5), and these correspond to two serotypes, Houston-1 and Marseille (15). Genotype I is more common in The Netherlands and Germany, whereas genotype II is more frequently observed in Switzerland, France, and Belgium (5, 8, 19, 23; S. Woestyn, N. Olivé, G. Bigaignon, and M. Delmée, unpublished data). Analysis of bacteremic cats showed a predominance of genotype II in The Netherlands and Germany (3, 20). On the basis of the observed opposite distribution of *B. henselae* variants in humans and in cats, it has been proposed that genotype I is more pathogenic for humans than genotype II. Implication of genotype I in atypical presentations of CSD such as osteomyelitis has never been studied before. Our present observations are in agreement with the hypothesis of a greater pathogenicity of genotype I *B. henselae*. However, this deserves further genotyping and clinical evaluation.

In conclusion, this is the first PCR detection of *B. henselae* genotype I from a processed MGIT culture medium observed in a case of CSD osteomyelitis.

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