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Case Report

Seronegative cat-scratch disease diagnosed by PCR detection of *Bartonella henselae* DNA in lymph node samples

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ABSTRACT

Cat scratch disease (CSD), the typical clinical manifestation of *Bartonella* infections usually follows a typical benign self-limited course. Nevertheless, a variety of unusual clinical manifestations and confusing imaging features can lead to misinterpretations and render the disease a diagnostic dispute. Routine laboratory tests exhibit varying reported sensitivity and are usually unhelpful in diagnosis, as serology fails in terms of specificity and/or sensitivity. Herein we report a case of seronegative *Bartonella* infection presenting as symptomatic suppurative lymphadenitis with abscess formation, which was surgically drained. Diagnosis was established by PCR analysis from lymph nodes samples obtained during the procedure. PCR detection of specific DNA fragments from lymph node biopsy provides a sensitive detection of disease. The technique should be considered for patients with suspected CSD and negative serology, since serological assays exhibit low sensitivity. In ambiguous cases, surgical exploration may provide tissue for diagnosis; it is well tolerated and affords improved recovery.

Introduction

History of animal contact, regional lymphadenopathy and constitutional symptoms comprise the typical presentation of cat scratch disease (CSD), an infectious disease caused nearly exclusively by *Bartonella henselae*, a Gram-negative coccobacillus. Most patients report recent contact with a cat, usually a kitten. The typical course is usually benign and self-limited and in most cases requires only supportive therapy. Imaging and serological studies in correlation with a clinical history of cat contact may facilitate the diagnosis and avoid unnecessary invasive procedures.

However, the imaging features of lymphadenopathy in CSD may be confusing.[1] In addition, a variety of other unusual localized or systemic clinical manifestations can lead in some occasions to misinterpretations[2,3] and render the disease a diagnostic dispute. Although serologic analysis is the most extensively evaluated minimally invasive diagnostic technique for the diagnosis of CSD, the sensitivity of serologic tests varies from one laboratory to another ranging from nearly 100% to < 30%.[4,5] Consequently, the disease can involve a prolonged and/or complicated course and further invasive diagnostic procedures can be required, as in our case.

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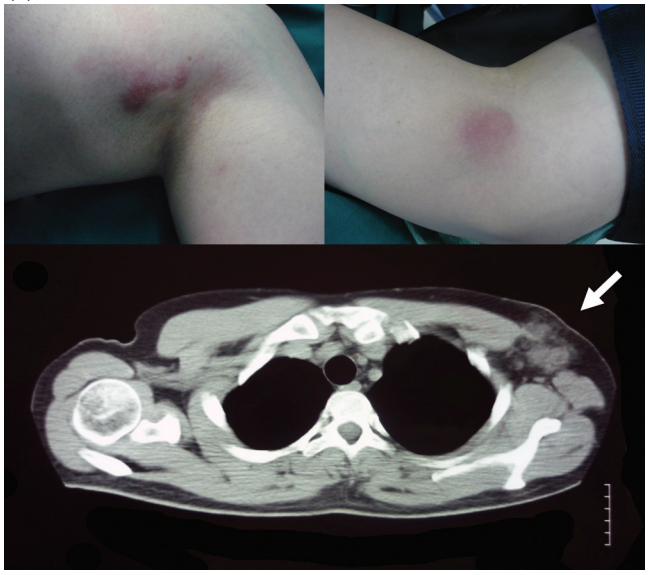
Case report

A 34-year-old Caucasian male patient was admitted to our hospital with pain and swelling in the left axilla and elbow, and a history of pyrexia for the past 2 months. The patient was an urban inhabitant, had a free medical history and was not receiving any medications. He denied promiscuous sexual behaviors or drug abuse. He was not a domestic animal owner and denied having been in contact with animals.

Upon physical examination, the patient's temperature was 38.4ºC. Two maculopapular erythematous skin rashes, with underlying tender and very painful palpable masses resembling lymphadenopathy were present at the axillary and the left epitrochlear regions (Fig. 1A). No scars or scratches were present at the left upper arm.

Laboratory work up showed normal hemoglobin, an elevated white blood cell count (15600 cells/mm^3; 68% neutrophils, 22% lymphocytes, 5% monocytes 1% basophils and 4% eosinophils). Liver enzymes were normal and C-reactive protein levels were 1.45 mg/L. Blood cultures and viral serologic test results were all negative. Moreover, serological tests were used to exclude *Chlamydia trachomatis*, EBV, CMV and HIV infection. Chest radiograph showed no particular findings. Computed tomography of the chest (Fig. 1B) revealed a well-enhanced soft tissue lesion over the left axillary region, measuring 4.0 x 3.5 cm (arrow), resembling a necrotizing lymphadenitis of the axilla with no signs of abscess formation.

(A)



(B)

Fig. 1 - (A) Photograph of the axillary and the left epitrochlear regions, showing the maculopapular erythematous skin rashes. Tender and very painful palpable masses were present at the underlying tissues resembling lymphadenopathy. (B) CT of the axillary region, showing a 4.0 x 3.5 cm soft tissue lesion at the left axilla (arrow), with no signs of abscess formation.

Cat scratch disease was suspected, but serological tests by means of in house indirect fluorescence assay (IFA) against *Bartonella quintana* and *Bartonella henselae* gave negative results. Despite the negative serology, azithromycin (500 mg the first day and 250 mg for the next four days) was administered. Regardless of antibiotic treatment the axillary mass lymphadenopathy showed no signs of resolution, while the laboratory inflammatory signs and fever persisted for the following 5 days.

Surgical exploration of the lesions was resorted to as a diagnostic procedure and revealed two abscesses at the epitrochlear and axillar region, respectively. Both abscesses were present within a cluster of enlarged lymph nodes, they were both drained, and pus was sent for culture. A sample of lymph nodes was sent for histological examination and PCR assay.

Pathological findings demonstrated granulomatous inflammations with stellate necrosis suggesting CSD as one of the possible diagnosis (Fig. 2). Culture results were negative for microbial growth. Gram staining and Warthin-Starry silver staining were also negative. The final diagnosis was established by lymph node samples sent for PCR analysis (16S rRNA gene amplification), which revealed the presence of *B. henselae* DNA in the specimen.

Postoperative, the high-grade fever resolved spontaneously and the laboratory findings showed remission of the inflammatory signs. The patient was discharged on the second postoperative day. No additionally work up was performed during his follow-up and two months later the patient was considered cured.

(A) (B)

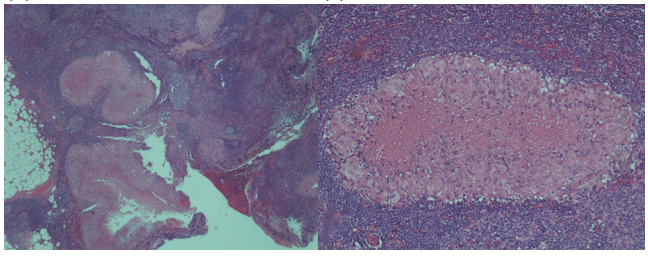


Fig. 2 - (A) Lymphadenitis with central necrosis and neutrophilic aggregation. (B) Higher-power view of necrotic granuloma in axillary lymph node, rimmed by epithelioid histiocytes, multinucleated giant cells, lymphocytes and eosinophils. (Haematoxylin and eosin staining, x100).

Discussion

Culture of *Bartonella* spp. from human specimens is very difficult. Therefore, clinical suspicion for CSD is usually confirmed serologically by the detection of antibodies against *B. henselae* or by the detection of *Bartonella* DNA in affected tissue.

Serological testing (mainly IFA) is the first and most practical diagnostic tool towards laboratory detection of suspected CSD,[5] although it exhibits variability, as many

different tests are available. The sensitivities of different IFAs range from 14% to 100%, depending on the antigen used, the cut-off chosen, and the test procedures.[5-7]

An IgM titer of 1:16 or higher indicates acute disease, with 3-month duration of detection in 50% of patients.[6,8,9] Thus, IgM antibodies are infrequently detected in serology and negative results do not rule out acute disease. An IgG titer higher than 1:256 is considered evidence of current or past *Bartonella* infection. Titers of 1:64 to 1:128 are considered equivocal. IgG titers also decrease with time; 75% of patients become seronegative after 1 year. There are reported decreases in IgG titer even after 4 weeks in some cases.[5,10] However, when IgG antibodies persist for up to a year, it is difficult to differentiate and diagnose active infection versus previous exposure to the bacterium.

Evidence shows that some patients never mount a detectable antibody response and that 88% of patients suspected of having CSD have detectable antibody, versus 3% of healthy controls.[11] Thus, disadvantages of serologic diagnosis include variable sensitivity and specificity and inability to distinguish between active and prior infection.[12,13] In addition, due to the lack of *Bartonella* species-specific antibody response, cross-reactivity between different *Bartonella* spp., Epstein-Barr virus, cytomegalovirus, *Toxoplasma gondii* and *Streptococcus pyogenes* may occur.[12,14,15]

Concerning histopathological examination of affected lymph nodes to determine the cause of lymphadenopathy, there is no specific pathology suggestive for *B. henselae* infection.[12] Even when Warthin-Starry silver stain is used to identify the causative bacterium, histopathologic findings are strongly suggestive for CSD but not definitive.[16] Nevertheless, histopathologic examination of affected lymph nodes is crucial when other malignant or granulomatous diseases (such as brucellosis, tuberculosis, lymphogranuloma venereum, histoplasmosis or coccidioidomycosis) and HIV infection have to be ruled out.[17]

Advanced diagnostic techniques such as PCR on lymph node or other material have been applied to the detection of *Bartonella*. Detection of *B. henselae* DNA in blood may prove useful, especially in cases where lymphadenectomy or biopsy is not feasible or serological results are ambiguous. PCR provides the advantages of high specificity and rapid identification, however lacking in sensitivity, ranging from 43% to 76%.[12,18,19] The sensitivity of PCR with samples of lymph node tissue or aspirates is 30-60% for CSD. PCR amplification can be also performed with pus samples drawn from lymph nodes with a reported sensitivity between 58 and 96%, therefore a lymph node biopsy can be avoided.[20-23] However, pus can only be collected from approximately 15% of the patients as 10 to 35% of the infected nodes progress to suppuration.[20,24]

In our case, despite the fact that the patient denied having been in contact with animals, clinical suspicion regarding CSD was raised. Nevertheless, IFAs were not helpful in establishing the diagnosis. The CT imaging resembling a soft – tissue necrotizing infection and lymphadenopathy was also not typical for *Bartonella* infection.[5] It has been proposed that a minority of patients with cat-scratch disease may actually require surgical drainage of a symptomatic abscess and lymph node sampling. Between 10 and 35% of the infected nodes progress to suppuration and evacuating the pus is necessary in this condition.[24] Suppurative tense and painful nodes should be drained, while incision of non-suppurative lesions should be avoided, as chronic draining fistulae or compromised healing may result.[4,5,25,26] As the clinical status of our patient was not improving despite antibiotic treatment, the use of an invasive approach was rendered necessary. Surgical exploration revealed the abscesses not clearly depicted by the CT. Despite the reported low sensitivity, the final diagnosis in our patient was established by PCR.

In cases where manifestations of CSD as lymphadenopathy or abscesses are self-limited, most patients have gradual resolutions of symptoms even without antibiotic treatment. Azithromycin treatment can be considered for patients with significant lymphadenopathy. In complicated *Bartonella* infections on immunocompromised patients, there is a dramatic response to antibiotics. Thus, seriously ill immunocompetent individuals are treated with similar regimens despite the lack of data.[12,27]

In conclusion, despite advances in diagnostic means, the diagnosis of CSD may still be challenging. Cat scratch disease should be considered in patients with chronic (> 3 weeks) lymphadenopathy. Given the low sensitivity of the serological assays, PCR analysis for *B. henselae* should be considered for patients with suspected CSD and negative serology. In ambiguous cases like ours, careful surgical exploration may provide tissue for diagnosis. It is well tolerated, and affords improved recovery with minimal complications.

Conflict of interest

All authors declare to have no conflict of interest.

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