A case of granulomatous hepatitis due to *Bartonella henselae* Infection

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Abstract:

Background:

Bartonella Henselae (*B. henselae*) is a rare cause of granulomatous hepatitis. The diagnosis of hepatic bartonellosis is difficult to establish due to its fastidious nature, lack of viable species on special stains, and non-specific findings on histopathology. Once the diagnosis has been established little is known of the optimal treatment.

Case:

We present a case of hepatic bartonellosis in an immunocompetent woman who presented with right upper quadrant pain and a five cm right hepatic lobe mass on CT scan. The patient underwent a right hepatic lobectomy. The surgical pathology revealed florid necrotizing granulomatous hepatitis favoring an infectious etiology. However, despite extensive histological and serological evaluation a definitive diagnosis was not established. Thirteen months later the diagnosis of hepatic bartonellosis was established on the basis of PCR of the surgically removed liver tissue. Interestingly, despite three separate courses of antibiotic therapy, there was evidence of chronic infection on specific enrichment culture of venous blood and histological features of persistent granulomas.

Conclusion:

The diagnosis of hepatic bartonellosis is exceedingly difficult to establish and mandates a high degree of clinical suspicion. Advanced diagnostic approaches may be required in select patients to make the diagnosis. Once the diagnosis has been established, little is known of the optimal antimicrobial therapy and close follow up is needed to ensure complete eradication.

Key Words: granulomatous hepatitis, *Bartonella Henselae*, diagnosis, treatment
Background

Classical cat scratch disease (CSD) is caused by *B. henselae*, a fastidious, aerobic, Gram negative proteobacterium that is transmitted by the bite or scratch of a cat. In addition to its natural feline host, *B. henselae* has been isolated from dogs and organism-specific DNA sequences have been amplified from insects, including the flea *Ctenocephalides felis*. The typical symptoms of hepatic bartonellosis in immunocompetent human subjects include right upper quadrant pain, fevers, malaise, weight loss, chills and headaches. Hepatic bartonellosis has been reported in 1-2% of CSD cases, and represents the third most common clinical manifestation after fever and lymphadenopathy.

The diagnosis of hepatosplenic *B. henselae* infection is being recognized more frequently as a result of improved liver imaging studies and the availability of more sensitive and specific molecular microbiological diagnostic techniques. Unenhanced CT scans typically show lesions that are hypoattenuating relative to the normal liver parenchyma. Contrast-enhanced studies may reveal hypoattenuation, iso-attenuation, or rim enhancement relative to the surrounding, uninvolved liver. On MR imaging, the granulomas appear as low-signal-intensity nodules on T1-weighted images, and as high signal-intensity nodules on T2-weighted images. Peripheral enhancement may be seen on gadolinium-enhanced, T1-weighted images. In some cases, only nonspecific imaging abnormalities are present.

Currently there is no “gold standard” for the diagnosis of bartonellosis or chronic *Bartonella* sp. bacteremia. The traditional diagnostic criteria for CSD included (1) contact with a cat and history of a scratch or other inoculation event, (2) positive cat scratch skin test reaction, (3) regional
lymphadenopathy with no other apparent etiology, and (4) characteristic histopathologic features on biopsy. More recently, Hansmann et al. demonstrated that the detection of B. henselae DNA by PCR methods added to the diagnostic sensitivity of the traditional criteria.

Necrotizing granulomas are the histologic hallmark of B. henselae hepatitis. They are due to the focal accumulation of activated macrophages, with a surrounding rim of lymphocytes and fibroblasts. However, hepatic granulomas are nonspecific and relatively common, occurring in up to 15% of cases in large biopsy series and in multiple disease settings. Steiner silver stains have historically been the preferred method of detection of B. henselae. However, a recent study comparing silver stains to immunohistochemistry (IHC) and PCR revealed poor agreement among the three tests. The findings reflected the limited sensitivity of IHC and silver stains.

Conventional bacterial cultures of liver tissue are rarely diagnostic, due to the fastidious nature of the bacterium. In order to overcome the low sensitivity of bacterial culture assays, recent studies have focused on the use of PCR assays to detect B. henselae target genes including 16S rRNA, 16S-23 ITS region, gltA, ribC and htrA. Anderson et al reported a sensitivity of 84% using a Bartonella htrA gene target in patients clinically suspected of having classical CSD. In comparison to antibody-based methods, PCR assays do not rely on the presence of a humoral immune response, and may therefore be diagnostic at an earlier stage of infection, or during chronic infection when the patient may be anergic. Furthermore, PCR testing avoids the problem of antibody cross-reactivity that has been observed between B. henselae and other bacterial species including Coxiella burneti and non-B. henselae Bartonella strains. More recently, Breitschwerdt and colleagues developed an optimized enrichment culture method that enhances diagnostic detection and molecular typing (by DNA sequencing of the PCR amplicon) of B. henselae and other Bartonella spp. from venous blood. This method combines the use...
of a specialized culture medium and prolonged incubation (up to 14 days) in a high CO\textsubscript{2} environment with PCR testing and DNA sequencing of amplicons obtained at various stages during the testing platform. This highly sensitive method is able to detect active infection in patients who are lack \textit{B. henselae} antibodies and in whom conventional bacterial cultures have been negative\textsuperscript{11,12}.

\textit{B. henselae} antibody testing has been reported to have a higher yield as compared to conventional bacterial culture, and is considered a useful diagnostic tool. Early studies by Regnery et al described an indirect fluorescent-antibody test with a sensitivity and specificity of 88 and 94\% respectively. However, this study population consisted of patients suspected of CSD\textsuperscript{13}. Further, up to 50\% of bacteremic patients do not mount measurable antibody titers against the infecting \textit{B. henselae} strain\textsuperscript{14}. This may be partly due to technical limitations of the different antibody assays (ELISA, EIA, IFA, commercial vs. in house assays), the effect of sample handling, subjective interpretation of test results, variations in \textit{B. henselae} seroprevalence in different geographic regions\textsuperscript{15,16}, and the genetic heterogeneity of different bacterial strains\textsuperscript{9,10,12}.

Treatment of \textit{B. henselae} infections is dependent upon the clinical disease, the status of the host immune system and the severity of the illness. Historically, CSD has represented the most common clinically recognized scenario and was thought to inevitably have a self-limited course. However, recent studies from Israel suggest that a subset of CSD patients develop chronic illnesses, characterized by musculoskeletal manifestations\textsuperscript{17}. Several retrospective studies have failed to demonstrate any significant benefits of antibiotic therapy in CSD. Bass and colleagues performed the only prospective clinical trial, a double blind, placebo-controlled study of azithromycin treatment of immunocompetent hosts\textsuperscript{18}. They reported a significant decrease in lymph node size after 7 to 14 days of antibiotic therapy (\textit{P} = 0.026). However, azithromycin did not prevent the progression to disseminated disease or the
development of infectious complications. Recently, rapid resistance to azithromycin was demonstrated by in vitro passage of *B. henselae*\textsuperscript{19}.

Individuals with immune deficiencies and bacillary angiomatosis or peliosis hepatis (including HIV-infected patients) are thought to require a prolonged treatment course. Prolonged treatment (six to eight weeks) has also been recommended for patients with retinitis, chronic bacteremia, endocarditis, chronic lymphadenopathy and neurological disorders.

There is currently no consensus with regard to the antibiotic treatment of hepatic bartonellosis. In a retrospective study, Arisoy and colleagues successfully treated 19 pediatric patients with rifampin, alone or in combination with gentamicin\textsuperscript{20}. Combination therapy using doxycycline, erythromycin and azithromycin cleared *B. henselae*-induced granulomatous hepatitis in patients with graft infection following liver transplantation\textsuperscript{21,22}. The combination of doxycycline (100mg twice daily) with rifampin (300mg twice daily) has been used successfully to treat retinitis due to bartonellosis. Prednisone has been suggested as adjunctive therapy for hepatosplenic CSD that is unresponsive to antibiotics. However, immunosuppression should be used with caution, as it might contribute to the development of endocarditis\textsuperscript{23,24}.

*In vitro* testing has demonstrated sensitivity of *B. henselae* strains to a wide spectrum of antibiotics including beta-lactams, macrolides, cephalosporins, aminoglycosides, fluoroquinolones, doxycycline and rifampin\textsuperscript{25}. However, most of these agents are bacteriostatic and fail to eliminate the bacterium when used as monotherapy. As a result, recurrent bacteremia has been reported following treatment\textsuperscript{26,27}, even after a prolonged course of antibiotic treatment.
Surgical treatment has occasionally been reported in hepatosplenic *B. henselae* infection. Murano et al. described giant hepatic granuloma caused by *B. henselae* in a 10-year-old child that was treated by partial hepatic lobectomy.

**Case Report**

In August, 2008, a 36 year-old woman with no prior history of liver disease presented to her primary care physician complaining of abdominal pain of five days duration. The pain was constant, sharp, localized to the right upper quadrant, non-radiating, and associated with nausea but no vomiting. Review of systems was positive for fatigue. Initial laboratory testing revealed only a mildly elevated alanine aminotransferase (ALT) of 42 IU/L. A right upper quadrant ultrasound demonstrated hepatomegaly and fatty infiltration. Two weeks later, the patient presented to an outside hospital with worsening right upper quadrant pain, low-grade fevers, nausea and vomiting. An MRI of the abdomen revealed a two cm enhancing lesion of the right hepatic lobe. Fine needle biopsy of the lesion demonstrated a nonspecific, mixed inflammatory cellular infiltrate and steatohepatitis. Pathologic analysis of the sample was negative for malignancy. Fungal and mycobacterial cultures were negative. The patient was discharged home without a specific diagnosis and no treatment was recommended.

Two months later, the patient presented to Loyola University Medical Center with worsening right upper quadrant pain and fever. An abdominal CT scan revealed a 4.8 x 4.7cm mass lesion involving the right hepatic lobe that was thought to be suspicious for malignancy (Fig. 1). In January of 2009, the patient underwent a partial right hepatectomy with excision of the mass. Histopathological studies of the resected specimen showed florid necrotizing granulomatous inflammation with pseudotumor formation (Figure 2). Immuno-histochemical stains for mycobacteria, fungal organisms, and cytomegalovirus were
negative. The patient’s presenting symptoms resolved, and she was discharged home after an uneventful postoperative recovery.

Two months later, the right upper quadrant pain and low-grade fevers recurred. The patient presented to an outside hospital where an extensive evaluation was initiated (Table 1). Serologies for acute viral hepatitis were negative. Qualitative antibodies for toxoplasmosis, human immunodeficiency virus, and *Entamoeba histolytica* were negative. An EIA for *Borrelia burgdorferi* antibodies was negative. IFA assays for *Bartonella henselae* and *Bartonella quintana* were negative. Quantitative urinary antigens for histoplasmosis and blastomycosis were negative, and a quantitative serum antigen for cryptococcus was negative. Skin testing for tuberculosis and quantiferon gold were negative. A random CT guided needle liver biopsy showed mild macro- and micro-vesicular steatosis and non-specific chronic inflammation. Repeat special stains, fungal and mycobacterial cultures were negative. The patient’s symptoms improved, and she was discharged home on analgesics.

Two months later, the patient represented to the same outside hospital with recurrent right upper quadrant pain, nausea, and fevers of up to 102.4°F. An abdominal CT revealed several low attenuation lesions involving both segments of the liver, with the largest one measuring 4.1 x 2.9cm. The patient was transferred to Loyola University Medical Center for further evaluation. A detailed travel and exposure history to ticks, rodents or other vectors of unusual infectious etiologies was unrevealing. On physical examination, the patient was afebrile. There was no lymphadenopathy or hepatosplenomegaly. Her right upper quadrant was tender to palpation without rebound tenderness or guarding. Laboratory analysis revealed an aspartate aminotransferase (AST) of 93 IU/L UNITS, ALT of 74 IU/L, alkaline phosphatase of 193 IU/L, and total bilirubin of 0.6 mg/dL. The hemoglobin was 13.4 gm/dL, and the WBC count was 4.9 K/UL with a normal differential. Bacterial blood cultures, urinalysis,
stool cultures, and stool testing for *Clostridium difficile* were negative. A chest radiograph showed no infiltrates or lymphadenopathy. Antinuclear antibody testing was positive at low titer (1:40), and antimitochondrial antibody testing was negative.

On the third day of her hospitalization, the patient developed a temperature of 101.1°F. A comprehensive investigation for fever of unknown origin was initiated (see Table 1). Antibody titers for *Bartonella quintana*, and *Brucella* sp. were negative. An RPR was negative. Repeat HIV antibody testing was negative. A peripheral smear for malaria was negative. A transesophageal echocardiogram did not reveal any valvular vegetations. The patient refused a lumbar puncture. A percutaneous liver biopsy was performed and revealed extensive granulomatous hepatitis with occasional fibrin rings, with a background of mixed, micro- and macro-vesicular steatosis. Based on the presence of fibrin rings, the possibility of Q fever was entertained. However, ELISA testing for *Coxiella burnetti* antibodies was negative. Special stains and cultures for AFB, fungal organisms, and cytomegalovirus were negative. Steiner silver stains for spirochetes and bacteria - including *Bartonella* sp.- were negative. The patient was empirically treated with a seven-day course of piperacillin and tazobactam. She remained afebrile for the remainder of her hospitalization. Based on the diagnosis of granulomatous hepatitis and the unrevealing workup for infectious organisms, the patient was started on empiric prednisone. Her liver enzymes, which had already been down-trending at the start of prednisone treatment, completely normalized over the next several weeks. Her symptoms resolved, and she was discharged home on a tapering dose of prednisone.

Based on the striking features of her hepatic granulomas, the possibility of hepatic Bartonella infection was raised, despite the negative Bartonella antibody titers. A sample of formalin-fixed liver tissue from the initial liver resection was sent for *B. henselae* PCR testing at the University of Arkansas. A positive
signal targeting a 153 bp fragment of the 16S rRNA gene was obtained, suggesting hepatic *B. henselae* infection. Upon further questioning, the patient reported that she had intermittently come in contact with a cat while visiting her mother’s house. However, she did not recall any scratches, bites or contact with fleas.

Based on a presumptive diagnosis of hepatic bartonellosis, the patient was started on azithromycin 250mg daily, and her prednisone was discontinued. Two weeks later, the patient developed diarrhea and abdominal cramping that were attributed to her antibiotic. Azithromycin was discontinued, and the patient was started on a nine-week course of clarithromycin at a dose of 500mg twice daily. Her symptoms completely resolved.

Three months later, her right upper quadrant pain recurred, and she presented to our emergency room once again. On examination, she was afebrile and her vital signs were stable. There was no jaundice. Right upper quadrant tenderness was present in the area of the excisional scar. There was no palpable hepato-splenomegaly. No skin rashes were present. AST and ALT levels were elevated to 109 IU/L and 75 IU/L, respectively. The bilirubin, alkaline phosphatase, and INR were normal. A liver-protocol CT revealed a 2.4 cm, low density lesion at the previous surgical site. This finding was felt to be nonspecific and due to scarring from previous surgery. However, given her recurrent symptoms, she was empirically treated with a six-week course of ciprofloxacin 500mg twice daily.

An additional four months later, on a subsequent clinic visit, she reported improvement in her abdominal pain. However, the AST and ALT activities had risen to 139 IU/L and 195 IU/L, respectively. A transjugular liver biopsy was performed, and demonstrated small, scattered, non-caseating granulomas with a background of micro- and macrovesicular steatosis. A sample from the biopsy was sent to the
University of Washington for PCR analysis. A positive signal was obtained with a primer set directed against the \textit{B. henselae} ribC gene\textsuperscript{29}, again supporting the presence of \textit{B. henselae} DNA in the specimen. In order to determine whether the patient was bacteremic, venous blood samples were sent for specialized enrichment cultures, subcultures, and PCR analysis at the Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University. Conventional PCR targeting the 16S-23S intergenic spacer region followed by DNA sequencing revealed the presence of an SA2 strain of \textit{B. henselae} in a post-enrichment blood culture using a specialized growth medium (\textit{Bartonella} alpha Proteobacteria growth medium, BAPGM). As \textit{B. henselae} DNA was not amplified from the patient’s blood and serum prior to enrichment culture, the positive enrichment culture PCR result supported persistent bacteremia with viable \textit{B. henselae} organisms. By indirect fluorescent antibody (IFA) testing, the patient did not have antibodies at a 1:16 serum dilution to \textit{B. henselae}, \textit{B. koehlerae}, or \textit{Bartonella vinsonii} subsp. \textit{berkhoffii} genotypes I, II or III antigens.

\textbf{Conclusions}

Our case illustrates the challenges of establishing the diagnosis of \textit{B. henselae}-induced granulomatous hepatitis. Traditional diagnostic criteria for CSD, based on clinical features, may be absent. Steiner silver stains of infected liver tissue may not reveal the organism, and serologic tests may fail to detect \textit{B. henselae}-specific antibodies. Conventional bacterial cultures are unlikely to identify this fastidious organism. A high degree of clinical suspicion and the combination of optimized enrichment cultures and PCR amplification were required to unequivocally establish the diagnosis in our patient. Subsequently, we faced unexpected difficulties in eradicating our patient’s infection, as the patient’s hepatitis and
bacteremia recurred despite several, prolonged courses of antibiotic monotherapy. The optimal treatment of this infection remains to be defined, and careful post-treatment follow-up is recommended.

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Informed Consent:
Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

TV carried out the composition of the manuscript. CF assisted with editing and formatting of the manuscript, and served as the primary hepatologist. EB performed molecular analysis including PCR and DNA sequencing, and participated in editing of the final manuscript. CY provided pathologic images. JP assisted in anti-microbial therapy and monitored treatment response. All authors read and approved the final manuscript.


**Table 1**
Serological tests performed to identify the etiology of granulomatous hepatitis in the patient.

**Figure 1**
Abdominal CT image demonstrating a large mass lesion in the right hepatic lobe.

**Figures 2 and 3**
The right partial hepatectomy shows florid necrotizing granulomatous inflammation for a peudotumorous mass (Fig. 2, hematoxylin and eosin 100x). Necrotizing granulomatous inflammation with giant cells and characteristic palisading histiocytes (Fig. 3, hematoxylin and eosin 400x).

**Figure 4**
Repeat abdominal CT after initial hepatic resection. Several new, low-attenuation lesions are present in the right hepatic lobe. Similar, smaller lesions were present in the left hepatic lobe (not shown).
<table>
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