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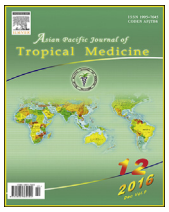

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Seronegative disseminated *Bartonella* spp. infection in an immunocompromised patient

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ABSTRACT

An 11 year old, hispanic girl with a history of B-cell acute lymphoblastic leukemia was admitted to the hospital for symptoms compatible with *Bartonella henselae* infection. The first molecularly diagnosed case of disseminated *Bartonella henselae* infection was reported in an immunocompromised patient in Lima, Peru. The analysis was confirmed by Polymerase Chain Reaction and automated sequencing of a liver biopsy sample, even though the serologic tests were negative. In conclusion, *Bartonella* spp. infection should have a particular diagnostic consideration in immunocompromised patients with fever of unknown origin and further investigation regarding the patient's past exposures with cats should also be elicited.

1. Introduction

Bartonella spp. is a genus that includes facultative, intracellular, gram-negative bacteria with the potential to cause prolonged intraerythrocytic bacteremia in mammals [1]. Analysis of the 16 s rRNA gene has allowed a phylogenetic reorganization of the genus identifying between 30 and 40 species [2,3]. The main species pathogenic to humans are *Bartonella henselae* (*B. henselae*), *Bartonella quintana* and *Bartonella bacilliformis* (*B. bacilliformis*), all of which are etiologies to a series of diseases such as Cat's Scratch Disease, Trench Fever, Chronic Lymphadenopathy, Endocarditis, Hepatic Peliosis and Carrion's disease [4].

We report an unusual presentation of *Bartonella* spp. infection with hepatic, splenic and renal involvement, diagnosed by PCR in a seronegative immunocompromised patient.

2. Case report

An 11 year old, hispanic girl with a history of B-cell acute lymphoblastic leukemia (B-ALL) came to the emergency department with a history of hyporexia, asthenia, nausea, vomiting, fever and myalgias that had begun 2 weeks earlier. The patient did not report any abdominal pain and neither rashes nor lymphadenopathies were evidenced during the exam. The patient's hometown is Tingo Maria in Huanuco, a rural central region in Peru. Her household had access to basic services and her family reported they had two cats, a dog, a hamster and a parrot at home. A month before presentation, the patient received induction chemotherapy IA for B-cell acute lymphoblastic leukemia with Cytarabine, Dexamethasone and Methotrexate; however, the induction IB was delayed due to a septicemia by positive *Klebsiella pneumonia* ESBL.

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On physical examination, the patient was wielding a Port catheter. She did not appear under distress and did not appear toxic. Further examination revealed an axillary temperature of 38.2 °C, blood pressure 110/65 mmHg, a heart rate 120 bpm. Examination of the lungs, heart and abdomen were normal. No lymphadenopathies, skin rashes or hepatosplenomegaly were evident.

A complete blood count evidenced leukocytosis with 22.21×10^3 cells/mL and 90% neutrophils. She had a hemoglobin of 9.5 g/dL and her platelets were at 299×10^3. C-reactive protein was elevated at 13.46 mg/dL. Her liver function tests, creatinine and urea were within normal limits.

Blood and urine cultures were repeatedly negative during the patient's hospital stay. Blood samples cultured for fungi, and Tuberculosis were negative. Cultures from the Port catheter were ordered, but came back negative. Moreover, serologic tests for *Cryptococcus neoformans*, *Histoplasma capsulatum*, TORCH, Hepatitis B virus, HIV and *B. henselae* were also negative.

An abdominal Computerized tomography (CT) was ordered revealing hypodense lesions in the liver, spleen and kidneys (Figure 1A, C, E). The lesions in the liver measured 3.3 cm in segment VI and 2.1 cm in segment VIII as opposed to the diffuse distribution of the lesions found in the spleen and kidney that measured between 0.9 cm and 1.6 cm. The CT also revealed bilateral axillary adenopathies.

A liver biopsy was ordered and the results showed a filiform distortion with a complete disruption of the architecture composed of cicatricial tissue with both an acute and chronic inflammatory process. The liver samples were histochemically stained with Gram, Ziehl–Neelsen, Giemsa and periodic acid Schiff, all of which came back with negative results.

The biopsy specimen was sent to the Molecular Laboratory at the Institute of Nutritional Research in Lima–Peru for a molecular study. DNA was extracted from the sample and the 16S rRNA gene from *Bartonella* spp. was amplified following the conditions described by del Valle *et al* [13]. The results were positive and the diagnosis of disseminated *Bartonella* spp. infection was established (Figure 2). Henceforth, the antibiotic treatment with Vancomycin, Meropenem and Caspofungin, initiated based on a clinical suspicion of bacteremia or fungemia, was suspended and replaced with a daily course of Azithromycin.

The patient's clinical condition improved gradually with the new antibiotic regimen. The fever decreased and the patient no longer reported myalgias, nausea or vomiting, even though hyporexia persisted. A follow-up Abdominal CT scan was

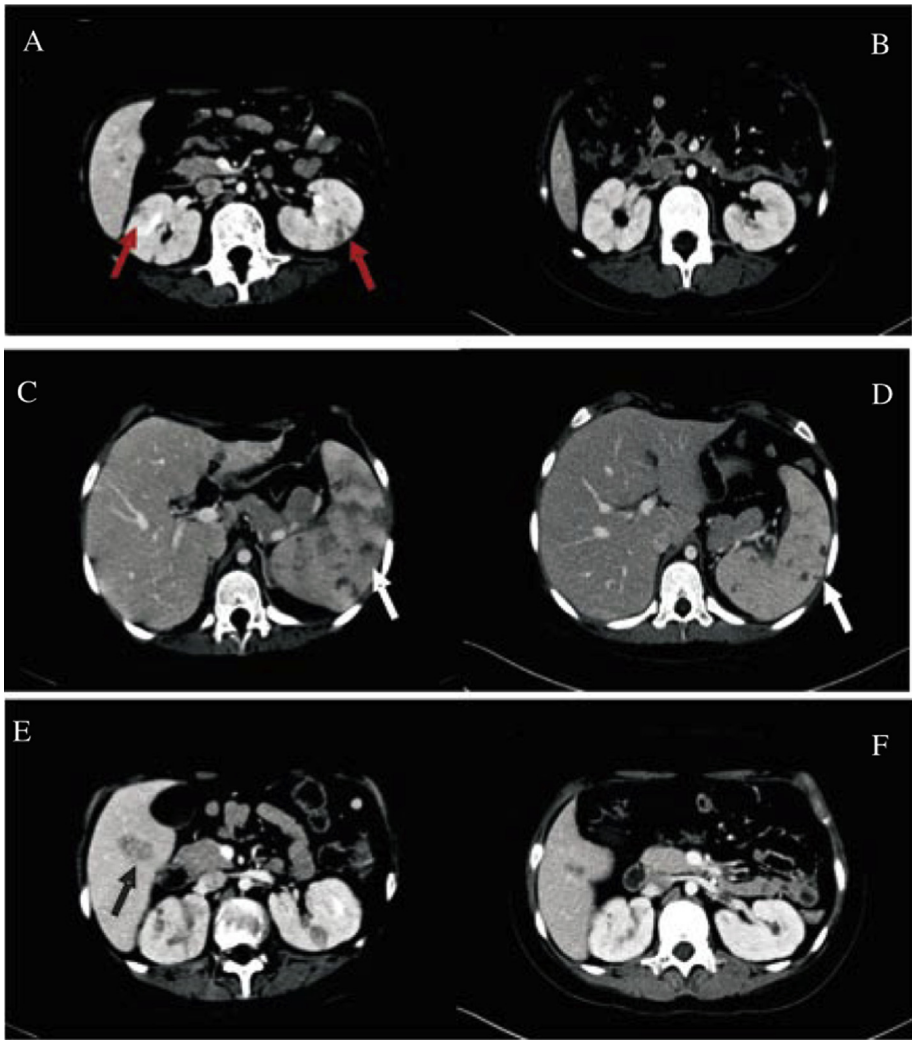

Figure 1. Computer tomography before and after the treatment.
Transverse view showing nodular hypodensities measuring between 9 and 16 mm in the spleen and kidneys (A with red arrows, and C with white arrow). The largest hypodensity is seen in the liver measuring 33 mm (black arrow, E). Most of the lesions in the spleen and kidneys remitted 2 months after treatment (B, D, F). The hepatic lesion decreased in size (F).

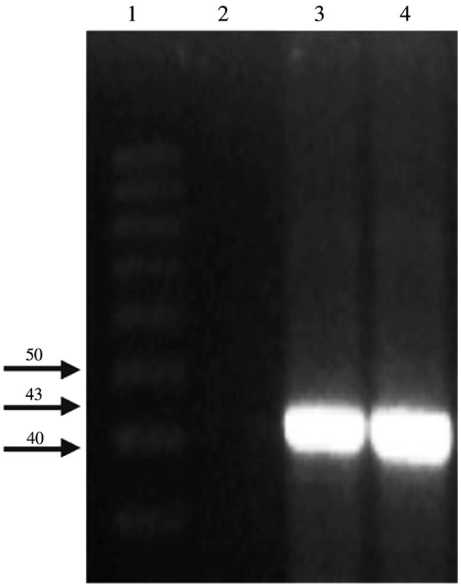

Figure 2. Conventional PCR of 16S *Bartonella* spp. for the patient's sample.
The amplified product is 438 base pairs (bp). (1) Molecular weight marker. (2) Negative control. (3) Positive control. (4) Sample.

ordered after a 26 day-course of antibiotic treatment with Azithromycin had begun and it showed a significant reduction in the size and number of the lesions in the liver, spleen and kidneys (Figure 1). The patient continued with a favorable clinical course. Eventually she was discharged with stable clinical conditions and was later followed up to continue the induction therapy for LLA-B.

3. Discussion

Bartonella spp. are responsible for a worldwide distribution of zoonotic infections [5]. The clinical course varies from a self limited infection by *B. Henselae* [6], to fatal cases seen in patients infected by *B. bacilliformis* where mortality rates can reach 85% if left untreated [7]. In Peru, the incidence of *B. bacilliformis* infection is 12, 7/100 person-years, and it increases to 38/100 person-years in children below the age of 5 [8]. *B. henselae* infection is also reported and it mainly affects the population below the age of 21 [9], although no local studies on prevalence have been published.

The main reservoir for *B. henselae* is the domestic cat with asymptomatic bacteremia even though transmission has also been reported from dogs [10,11]. On the other hand, humans are the only reservoir for *B. quintana* and *B. bacilliformis* [3]. The transmission for most of the species in the Bartonella genus is through direct contact or via insect vectors. *B. henselae* can be transmitted by a cat's scratch, bite or cat flea [12], as is suspected for our patient's case due to the history of two household cats. In contrast, transmission of *B. bacilliformis* requires a phlebotomus mosquito of the genus Lutzomyia [12]. *Bartonella* spp. infects erythrocytes and vascular endothelial cells by means of a flagellum causing intraerythrocytic bacteremia [13]. *B. henselae* aids this process by inhibiting lysosomal fusion and acidification after it has been phagocyted [12].

Bartonella spp. infections have a more disseminated presentation in immunocompromised patients as reported in cases with HIV infection [14], LES [15], oncological diagnoses [16] or post-transplanted individuals [17–20]. These patients generally have a dermatologic component with violaceous cutaneous lesions and a visceral component with bacillary angiomatosis [21–23]. Furthermore, bacteremia, bacillary peliosis, splenitis and osteomyelitis can also develop [24,25].

In our case report, the patient had a diagnosis of ALL-B and her immunity was further suppressed with induction therapy. This may explain the resulting hepatosplenic and renal involvement.

The gold standard for the diagnosis of *B. Henselae* is serology [26]. Assays for *B. Henselae* can detect IgM and IgG and may support the clinical diagnosis. Sensitivities vary between 14% and 100% for IgG and 2%–50% for IgM whereas the specificities are 34%–100% and 86%–100% respectively [6].

Cross-reactivity with other microorganisms may contribute to false positives and also, these tests may be inaccurate in immunocompromised patients due to a diminished antibody response [27]. The latter may explain the persistent seronegativity evidenced in our patient. Amplification of the 16s rRNA Bartonella gene via PCR is an alternative molecular diagnostic test with higher sensitivities and specificities reported [4]. In our seronegative patient, this method was essential for the etiologic diagnosis of *Bartonella* spp. infection under the suspicion of fever of unknown origin, and for the consequent adjustment of treatment suspending unnecessary antibiotics.

IDSA guidelines recommend treatment with Azithromycin for cat scratch disease affecting skin and soft tissue [28], however definite treatment for disseminated *Bartonella* spp. infection has not been established [29]. A cohort studied the efficiency certain antibiotics for Cat Scratch disease in immunocompetent patients [30] but to our knowledge a study evaluating antibiotic efficiency for disseminated *Bartonella* spp. infection in immunocompromised patients, has not been published. Appropriate treatment is paramount in these patients due to the likelihood of a complicated disseminated infection that may lead to a critical condition or death.

This study presents the first reported case in Peru of molecular identification of *Bartonella* spp. via PCR of a hepatic tissue specimen from an immunocompromised patient diagnosed with LLA-B and seronegative on multiple occasions for *B. henselae*.

In conclusion, *Bartonella* spp. infection should have a particular diagnostic consideration in immunocompromised patients with Fever of Unknown Origin and further investigation regarding the patient's past exposures with cats should also be elicited. In this case, the definitive diagnosis was made by a molecular technique after serology came back negative, stressing the importance of PCR in a complicated and rare presentation of *Bartonella* spp. infection. Prolonged treatment with Azithromycin alone proved effective in this patient, however there is a need for consensus on the treatment for immunocompromised patients.

Conflict of interest statement

We declare that we have no conflict of interests.

References

[1] Siamer S, Dehio C. New insights into the role of Bartonella effector proteins in pathogenesis. *Curr Opin Microbiol* 2015; **23**: 80-85.

[2] Kosoy M, Hayman DT, Chan KS. Bartonella bacteria in nature: where does population variability end and a species start? *Infect Genet Evol* 2012; **12**(5): 894-904.
[3] Angelakis E, Didier R. Pathogenicity and treatment of Bartonella infections. *Int J Antimicrob Agent* 2014; **44**(1): 16-25.
[4] del Valle Mendoza J, Silva Caso W, Tinco Valdez C, Pons MJ, del Valle LJ, Casabona Oré V, et al. Diagnosis of Carrion's disease by direct blood PCR in thin blood smear negative samples. *PLoS One* 2014; **9**(3): e92283.
[5] Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. *J Vet Emerg Crit Care* 2010; **20**(1): 8-30.
[6] Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. *Pediatrics* 2008; **121**(5): e1413-e1425.
[7] Maguina C, Garcia PJ, Gotuzzo E, Cordero L, Spach DH. Bartonellosis (Carrión's disease) in the modern era. *Clin Infect Dis* 2001; **33**(6): 772-779.
[8] Chamberlin J, Laughlin LW, Romero S, Solórzano N, Gordon S, Andre RG, et al. Epidemiology of endemic *Bartonella bacilliformis*: a prospective cohort study in a Peruvian mountain valley community. *J Infect Dis* 2002; **186**(7): 983-990.
[9] Lamps LW, Scott MA. Cat-scratch disease: historic, clinical, and pathologic perspectives. *Am J Clin Pathol* 2004; **121**(Suppl): S71-S80.
[10] Keret D, Giladi M, Kletter Y, Wientroub S. Cat-scratch disease osteomyelitis from a dog scratch. *J Bone Jt Surg Br* 1998; **80**(5): 766-767.
[11] Rojas N, Troyo A, Castillo D, Gutierrez R, Harrus S. Molecular detection of *Bartonella* species in fleas collected from dogs and cats from Costa Rica. *Vector Borne Zoonotic Dis* 2015; **15**(10): 630-632.
[12] Mosepele M, Mazo D, Cohn J. Bartonella infection in immunocompromised hosts: immunology of vascular infection and vasoproliferation. *Clin Dev Immunol* 2012; **2012**: 612809.
[13] Minnick MF, Mitchell SJ, McAllister SJ. Cell entry and the pathogenesis of Bartonella infections. *Trends Microbiol* 1996; **4**(9): 343-347.
[14] Favacho A, Roger I, Akemi AK, Pessoa AA Jr, Varon AG, Gomes R, et al. Molecular identification of *Bartonella henselae* in a seronegative cat scratch disease patient with aids in Rio de Janeiro, Brazil. *Rev Inst Med Trop Sao Paulo* 2014; **56**(4): 363-365.
[15] Vargas-Hitos JA, Sabio JM, Navarrete-Navarrete N, Arenas-Miras Mdel M, Zamora-Pasadas M, Jiménez-Alonso J. Cat scratch disease in an immunosuppressed patient with systemic lupus erythematosus. *Lupus* 2016; **25**(3): 310-311.
[16] Mazur-Melewska K, Jończyk-Potoczna K, Mania A, Kemnitz P, Szydłowski J, Służewski W, et al. The significance of *Bartonella henselae* bacterias for oncological diagnosis in children. *Infect Agent Cancer* 2015; **10**: 30.
[17] Psarros G, Riddell J 4th, Gandhi T, Kauffman CA, Cinti SK. *Bartonella henselae* infections in solid organ transplant recipients: report of 5 cases and review of the literature. *Medicine (Baltimore)* 2012; **91**(2): 111-121.
[18] Gai M, d'Onofrio G, di Vico MC, Ranghino A, Nappo A, Diena D, et al. Cat-scratch disease: case report and review of the literature. *Transpl Proceed* 2015; **47**(7): 2245-2247.
[19] Rostad C, McElroy AK, Hilinski JA, Thompson MP, Drew CP, Denison AM, et al. *Bartonella henselae*-mediated disease in solid organ transplant recipients: two pediatric cases and a literature review. *Transpl Infect Dis* 2012; **14**(5): E71-E81.
[20] Picascia A, Pagliuca C, Sommese L, Colicchio R, Casamassimi A, Labonia F, et al. Seroprevalence of *Bartonella henselae* in patients awaiting heart transplant in Southern Italy. *J Microbiol Immunol Infect* 2015; http://dx.doi.org/10.1016/j.jmii.2015.05.001.
[21] Moulin C, Kanitakis J, Ranchin B, Chauvet C, Gillet Y, Morelon E, et al. Cutaneous bacillary angiomatosis in renal transplant recipients: report of three new cases and literature review. *Transpl Infect Dis* 2012; **14**(4): 403-409.
[22] Psarros G, Riddell J 4th, Gandhi T, Kauffman CA, Cinti SK. *Bartonella henselae* infections in solid organ transplant recipients. *Medicine* 2012; **91**(2): 111-121.
[23] Orsag J, Flodr P, Melter O, Tkadlec J, Sternbersky J, Hruby M, et al. Cutaneous bacillary angiomatosis due to *Bartonella quintana* in a renal transplant recipient. *Transpl Int* 2015; **28**(5): 626-631.
[24] Chang C, Lee CJ, Ou LS, Wang CJ, Huang YC. Disseminated cat-scratch disease: case report and review of the literature. *Paediatr Intern Child Health* 2016; **12**: 1-3.
[25] Graveleau J, Grossi O, Lefebvre M, Redon H, Caignon JM, Pallardy A, et al. Vertebral osteomyelitis: an unusual presentation of *Bartonella henselae* infection. *Semin Arthritis Rheum* 2011; **41**(3): 511-516.
[26] Metzkor-Cotter E, Kletter Y, Avidor B, Varon M, Golan Y, Ephros M, et al. Long-term serological analysis and clinical follow-up of patients with cat scratch disease. *Clin Infect Dis* 2003; **37**(9): 1149-1154.
[27] La Scola B, Raoult D. Culture of *Bartonella quintana* and *Bartonella henselae* from human samples: a 5-year experience (1993 to 1998). *J Clin Microbiol* 1999; **37**(6): 1899-1905.
[28] Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the infectious diseases society of America. *Clin Infect Dis* 2014; **59**(2): e10-e52.
[29] Prutsky G, Domecq JP, Mori L, Bebko S, Matzumura M, Sabouni A, et al. Treatment outcomes of human bartonellosis: a systematic review and meta-analysis. *Int J Infect Dis* 2013; **17**(10): e811-e819.
[30] Margileth A. Antibiotic therapy for cat-scratch disease. *Pediatr Infect Dis J* 1992; **11**(6): 474-478.