A “Silent Culture-Negative” Abdominal Aortic Mycotic Aneurysm: Rapid Detection of Bartonella Species Using PCR and High-Throughput Mass Spectrometry

Matthew Koo; Sheri Manalili; Matthew J. Bankowski PhD; Rangarajan Sampath PhD; Steven A. Hofstadler PhD; and Joseph Koo MD

Abstract
A gram-negative, rod-shaped microorganism was detected in a 69-year-old man suffering from chronic back pain but otherwise exhibiting no signs of infection. The bacterium could not be identified using any routine diagnostic modality. A research use only application utilizing PCR and Mass Spectrometry was performed on nucleic acid extracted from the tissue sample. These studies resulted in the implication of Bartonella quintana as the underlying cause of the infection. B. quintana is not a well-known cause of an abdominal aortic mycotic aneurysm. This article will discuss the B. quintana infection, its diagnosis and treatment, and reinforce the potential of B. quintana as a possible etiology in mycotic aneurysms that show no apparent indications of infection. It will also explore the potential use of polymerase chain reaction detected by electrospray ionization mass spectrometry (PCR/ESI-MS) to help identify B. quintana in a situation where other conventional methods prove non-informative.

*This is a “research use only” method and is not approved for use in human clinical or in vitro diagnostic procedures.

Introduction
Bartonella quintana is a gram-negative, facultative intracellular parasite generally known for acting as an opportunistic pathogen. It is transmitted primarily through the bite of blood-sucking insects such as the human body louse Pediculus humanus corporis. Often infecting homeless individuals, it can infect immunocompromised patients—especially those afflicted with AIDS. Less frequently, it may infect immune-competent individuals. Recent studies indicate that in addition to lice, Bartonella vectors are much more numerous and diverse than previously understood; newly discovered vectors include various arthropods such as lice, fleas, and ticks. B. quintana is a well-known cause of diseases such as trench fever, endocarditis, and bacillary angiomatosis. While B. quintana has also been shown to be the underlying cause of chronic bacteremia, it is not widely regarded as a causative agent of a mycotic aneurysm. An extensive PubMed search inquiry of “mycotic aneurysm bartonella” did not yield any results. The diagnosis of this infection is usually difficult, even under ideal circumstances.

Case Presentation
A 69-year-old man with a past medical history of asthma, hypertension, and atrial fibrillation underwent an MRI of his spine to evaluate his chronic back pain. Although he does not own any pets, stray cats frequent his backyard and neighborhood, suggesting possible B. quintana exposure via an animal host. His MRI revealed a diffuse lumbar spondylosis and some degenerative disk changes with a Grade I anterior spondylolisthesis of L3 and L4. The MRI and sonogram showed a 4.6 cm infra-renal abdominal aortic aneurysm, extending into both common iliac arteries. He did not show any signs of fever, chills, abdominal pain, or any other symptoms of sepsis. He was well except for his chronic back pain. His CBC, ESR, basic metabolic profile, CRP, echocardiogram and physical examination did not reveal any signs of infection, specifically neither endocarditis nor angiomatosis. He underwent repair of the aneurysm, and the aortic tissue resected during the surgery was inflamed; the Gram stain and Warthin-Starry stains showed some gram-negative, rod-shaped microorganisms. Blood cultures taken after receiving pre-op cefazolin, given one gram intravenously every 8 hours for two days, showed no growth despite a prolonged period of incubation (i.e. two sets of blood cultures at 28 days). Extracted nucleic acid (MagnaPure) from the aortic mycotic aneurysm tissue was first subjected to direct 16S rRNA bacterial sequencing without success. The remaining nucleic acid, de-identified of all patient information, was shipped to a research laboratory in California (Ibis Biosciences) to process on the Ibis T5000™ Biosensor System, a research use only system. The Ibis T5000™ Biosensor System technology employs broad-range primers to amplify genetically conserved regions of known or unknown pathogens. The DNA amplicons are subsequently characterized by electrospray ionization time-of-flight mass spectrometry, which yields molecular weight measurements, from which base compositions (i.e. the number of A, G, C, and T nucleotides) are then derived. The Ibis T5000™ Biosensor System translates the base composition profiles in an automated fashion.

Two primer pairs from an 8-primer pair alpha proteobacteria panel were selected for this study. The assay targets information-rich regions of the bacterial genome amplified by PCR through an 8-primer pair panel designed to identify and broadly detect the species of alpha proteobacteria that may be present. The PCR amplicons were electrosprayed and analyzed on the Ibis T5000™ instrument at a rate of one PCR reaction per minute. The internally-calibrated mass spectra were converted to neutral mass measurements from which unique base compositions are assigned for bacterial identification. Amplification using primer pair 3569 targeting the citrate synthase gene resulted in a base composition of [A33, G28, C30, T31]. Primer pair 3575, targeting rpoB, the β subunit of the bacterial RNA polymerase, yielded a base composition of [A36 G20 C31 T25]. The base compositions from the two sets of primer pairs identified the organism as Bartonella quintana. Negative controls produced no amplicons, and internal calibrants were detected in both water controls and samples.

The patient’s sera was tested for both IgM and IgG Bartonella quintana and Bartonella henselae antibody and the results remained negative both at the approximate time of surgery and six weeks afterward. Except for the chronic back pain, the patient remained well following the surgery. He was not immunocompromised and tested negative for HIV infection. The patient was treated with three intravenous antibiotics: a 7-day course of gentamicin (80mg every 8 hours), six weeks of ceftriaxone (2g daily), and six weeks of doxycycline (100mg twice a day). Afterwards, the patient was administered oral doxycycline (100mg twice a day) for life.
Discussion

Bartonella quintana is a small, fastidious gram-negative rod well known to cause bacteremia, endocarditis and bacillary angiomatosis. In the presented case, the patient’s only manifestation of the infection was the formation of a mycotic aortic aneurysm, supported by the pathologic finding of the aortic tissue resected. As described above, the etiologic agent was later identified using a research use only PCR/ ESI-MS approach. B. quintana parasitizes endothelial cells and can induce angiogenesis of blood vessels. It has not been previously reported as a cause of a mycotic aneurysm formation, but it is strongly implicated as the etiologic agent in this case study. Among HIV-infected patients, B. quintana predominantly causes bacillary angiomatosis and endocarditis. In contrast, among immunocompetent hosts, B. quintana causes a multitude of symptoms resembling trench fever. The pattern of fever associated with a B. quintana infection can exist in the forms of a single febrile episode that lasts several days, many recurrent febrile episodes that last several days, or a persistent fever. The patient did not recall any form of febrile illness prior to the discovery of the mycotic aneurysm. He may have had a transient bacteremia followed by a “seeding” of the aorta by the B. quintana. Such a sequence of events causing infection could have potentially resulted in the formation of the mycotic aneurysm. His back pain decreased initially, but recurred after he recovered from the surgery. His back pain was most likely due to his vertebral degenerative arthritis rather than the dissection of his aortic aneurysm. This essentially caused his mycotic aortic aneurysm to appear “silent.”

Isolating the bacteria from a tissue or blood sample can make a definitive diagnosis of a B. quintana infection. However, in many cases, the isolation of this bacterium from culture remains difficult. In this particular case, we were unable to perform a culture from the sample because the specimen was placed in formalin prior to transporting it to the laboratory. Since there was no evidence of infection upon presentation, only histopathology was requested. Serology and direct 16S rRNA sequencing (i.e. PCR-based) was inconclusive. Since the antibody production in response to a B. quintana infection can vary, the serological results were not surprising. Patients with chronic B. quintana bacteremia (no infectious endocarditis) may present scanty or absent anti-Bartonella antibody response. In contrast, patients with B. quintana endocarditis usually exhibit high antibody titers. In the present case study, the patient exhibited undetectable IgG or IgM titers to B. quintana and B. henselae, even after six weeks. It should be emphasized that the patient did not have endocarditis clinically. In order to identify the rod shaped bacteria seen on the aortic tissue histopathology, nucleic acid was extracted and purified from the aortic tissue for 16S rRNA sequencing and PCR/ ESI-MS. The Ibis T5000™ Biosensor System has not been approved for clinical use; it is used to identify microorganisms in a research setting and can serve as a powerful tool in forensic analysis.

The drug of choice for a B. quintana infection is not fully established. In vitro susceptibility data has not correlated well with any specific clinical response. Doxycycline and macrolides can be used to treat this infection. Rifampin or gentamicin can also be added to doxycycline or a macrolide for increased efficacy. The optimal length of treatment depends on the type of disease involvement. If the disease involves B. quintana endocarditis, the accepted guide-