BARTONELLA-ASSOCIATED INFECTIONS

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Although descriptions of infections caused by Bartonella (formerly Rocheiimaec), such as Oroya fever, trench fever, and cat-scratch disease (CSD), have existed for more than 50 years, much of the current understanding of Bartonella-associated infections has resulted from the AIDS epidemic. In 1983, Stoler et al provided the first report of a Bartonella-associated infection in an HIV-infected individual. This report described an AIDS patient from New York who developed multiple subcutaneous vascular nodules that contained numerous bacillary organisms visualized by electron microscopy. Additional reports in the late 1980s described patients with similar cutaneous lesions, and these vascular proliferative lesions became known as bacillary angiomatosis (BA). During this time, however, investigators could not cultivate an organism from these lesions, and the cause remained unidentified.

In a December 1990 issue of the New England Journal of Medicine, separate groups of investigators respectively described three key new findings: (1) the close relationship of a DNA sequence from the bacillus in BA tissue to the agent of trench fever, Rocheiimaec quintana; (2) the isolation of a small, fastidious, gram-negative rod from patients with relapsing bacteremia; and (3) the association of a small bacillus with the unusual histopathologic entity of peliosis hepatitis in the livers of HIV-infected patients. These three research groups soon realized that their separate findings involved the same organism. This newly-recognized bacillary organism, which was subsequently named Rocheiimaec henselae, was initially presumed to be the sole agent of BA. In 1992, however, Keheler et

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al17 became the first group to isolate organisms from BA lesions, and in this process demonstrated that either \textit{R. quintana} or \textit{R. henselae} can cause BA.

The four species belonging to the genus of \textit{Rochalimaea} were moved to the genus of \textit{Bartonella} in 1993 after genetic studies showed the close relationship between the \textit{Rochalimaea} species and \textit{B. bacilliformis}, the original member of the genus \textit{Bartonella}.14 More recently, the genus of \textit{Grahamella} was merged with the genus of \textit{Bartonella}, and at present, nine cultivated species belong to the genus \textit{Bartonella}.11 Four of these species, \textit{B. bacilliformis}, \textit{B. henselae}, \textit{B. quintana}, and \textit{B. elizabethae}, have been documented to be pathogenic in humans. Four species, \textit{B. vinsonii}, \textit{B. hiramii}, \textit{B. taylorii}, and \textit{B. doshiae}, were isolated from small wild mammals, and \textit{B. claridgeae} was isolated from a domestic cat.3, 10, 26, 51 In addition, one group recently isolated a subspecies of \textit{B. vinsonii} from a dog with endocarditis.13

The spectrum of diseases caused by \textit{Bartonella} has rapidly expanded to include BA,47, 81 bacillary peliosis,76, 102 relapsing bacteremia,95, 98 endocarditis,28, 33, 90, 91 and “urban trench fever.”92 Moreover, in 1992, Regnery et al90 demonstrated antibodies to \textit{Bartonella} species in a panel of banked sera from immunocompetent patients with a clinical diagnosis of CSD, thus contradicting the initial belief that \textit{Aepifia felis} was the causative agent of CSD. Subsequently, \textit{B. henselae} was isolated from the lymph nodes of two patients with apparent CSD and \textit{B. henselae} DNA was detected by polymerase chain reaction (PCR) in specimens from patients with CSD.2, 31 In addition, the domestic cat was identified as the major reservoir for \textit{B. henselae}.45 Taken together, these data from 1992 to 1994 convincingly identified \textit{B. henselae}, not \textit{A. felis}, as the predominant causative organism of CSD.

\textit{Bartonella} species represent a fascinating group of emerging pathogens. This article presents a summary of the manifestations of human \textit{Bartonella} infections identified at present in North America and in Europe, but will not discuss \textit{B. bacilliformis}, an infection predominantly limited to South America. Although considerable knowledge of \textit{Bartonella}-associated infections has been gained, much data remain to be gathered to better define disease spectrum, prevalence, maintenance, and mode of transmission.

**CLINICAL MANIFESTATIONS**

**Immunocompromised Host**

**Bacillary Angiomatosis**

The manifestations of \textit{Bartonella} infection in the immunocompromised host are diverse and often nonspecific, frequently resulting in failure to diagnose these infections. The vascular proliferative lesions associated with \textit{B. henselae} and \textit{B. quintana} infection occur almost exclusively in HIV-infected and other immunocompromised individuals (although BA has been identified in seven apparently immunocompetent patients).75, 80, 93 \textit{BA} is usually a late-developing manifestation during the course of HIV infection, occurring after the CD4 count has decreased to less than 100 cells/mm\(^3\).44, 68 In one study of 42 immunocompromised patients with BA, the median CD4 count at the time of presentation was 21 cells/mm\(^3\).66 \textit{BA} has also been reported in immunosuppressed cardiac and renal transplantation patients,42, 81, 96 in addition to patients receiving chemotherapy71, 80 for hematologic malignancy.

The histopathologic changes of BA associated with \textit{Bartonella} infection have
been identified in many different tissues, including skin, brain, bone, lymph nodes, gastrointestinal tract, respiratory tract, and bone marrow. Cutaneous BA is the most frequently recognized manifestation, but can appear as diverse angiomatous lesions, including verrucous, papular, or pedunculated forms. Although these lesions most often have an erythematous base and a vascular appearance, they are occasionally dry, scaly, hyperkeratotic, or plaquelike. The lesions may occur singly or number in the hundreds, covering the entire body, usually enlarging if they remain untreated. Patients with BA can also present with subcutaneous nodules, with or without overlying tenderness and erythema, or with deep soft-tissue masses. Doppler ultrasonography often demonstrates the highly vascular characteristics of these deep soft-tissue lesions.

The angiomatous cutaneous lesions of BA can be indistinguishable clinically from those of Kaposi’s sarcoma (KS), particularly the verrucous form of KS. In addition, KS and BA can coexist in the same patient, further complicating the diagnosis of BA. Other entities to be considered in the differential diagnosis of cutaneous BA include pyogenic granuloma, verruca peruana (the cutaneous eruption associated with B. bacilliformis infection and seen only in the Andes of South America), and several types of subcutaneous tumors.

Focal BA affecting bone is usually extremely painful and manifests radiographically as a lytic lesion. The lytic lesions may occur singly, most commonly involving the radius, fibula, or tibia, and are easily identified by technetium methylene diphosphonate scanning. A cellulitic, tender, erythematous plaque overlying the osseous lesion has been identified in several patients. Many patients with osseous BA were diagnosed presumptively from biopsy of concomitant cutaneous BA lesions and by resolution of radiographically visualized bone lesions following appropriate antimicrobial therapy.

Lymphadenopathy frequently accompanies BA lesions, but the involved nodes rarely suppurate or drain. BA lesions of the gastrointestinal and respiratory tract can be visualized during endoscopy or bronchoscopy, respectively. Oral, anal, and peritoneal BA lesions have also been reported; in one of these patients a large angioma of the larynx enlarged and caused asphyxiation. Other infrequently encountered manifestations of focal Bartonella infection include BA of the bone marrow, brain parenchyma, intra-abdominal cavity, and cervix and vulva. Bartonella infection of the central nervous system has also been proposed as a cause of meningitis and neuropsychiatric deterioration in HIV-infected patients with Bartonella antibodies in the cerebrospinal fluid (CSF) and serum.

Bacillary Peliosis and Splenitis

In 1990, Perkocha et al first described bacillary peliosis hepatis. Peliotic changes of the liver (formation of venous lakes within the liver parenchyma) had been previously described in patients with terminal malignancy or after treatment with anabolic steroids; however, when the histopathologic changes of peliosis hepatis were observed at autopsy in a patient with cutaneous BA lesions, Warthin-Starry staining and electron microscopy were performed to look for bacilli in the liver tissue. Small bacilli were observed in the liver parenchyma adjacent to the peliotic spaces in this patient and then in seven additional HIV-infected patients. These eight patients had hepatomegaly; six had splenomegaly and two had cutaneous BA lesions. Other immunosuppressed patients have developed bacillary peliosis, including a patient who received chemotherapy for ovarian cancer.

By abdominal computed tomography, the peliotic spaces in liver and spleen
appear as hypodense lesions scattered throughout the organ parenchyma. Unfortunately, many infectious and malignant conditions, such as hepatic KS, lymphoma, Mycobacterium avium complex infection, and extrapulmonary pneumocystosis, can cause similar radiographic abnormalities.

Studies of the serum hepatic enzyme levels revealed that the alkaline phosphatase was the test most consistently elevated, by an average of 5-fold above the normal level and by as much as 10-fold in one patient. Aminotransferase levels were less dramatically elevated (average, two times normal). In this report, two of the patients with splenomegaly developed progressive pancytopenia. Other groups have reported thrombocytopenia or pancytopenia in association with peliosis hepatis and splenitis. Thus, the differential diagnosis of immunocompromised patients with hepatosplenomegaly and either thrombocytopenia or pancytopenia should include Bartonella infection.

Bacteremia and Endocarditis

HIV-infected individuals with BA or bacillary peliosis can present with concomitant Bartonella bacteremia. Bartonella bacteremia can also occur in the immunocompromised patient in the absence of focal bacillary infection. Slater et al described three immunocompromised patients (two infected with HIV and a third with pharmacologic immunosuppression following bone marrow transplantation) who developed B. henselae bacteremia. Prolonged and sometimes relapsing fever accompanied by weight loss and other constitutional symptoms were noted in these patients. Finally, endocarditis may complicate Bartonella infection in immunosuppressed patients, with development of valvular vegetations and a clinical course characteristic of subacute bacterial endocarditis. In the first reported case of Bartonella species endocarditis, Spach et al described an HIV-infected patient who presented with fatigue, night sweats, and weight loss. This patient had a holosystolic murmur, splenomegaly, mild renal insufficiency, and anemia. Echocardiography identified vegetations and regurgitant flow of both mitral and aortic valves. Bartonella quintana was isolated from blood cultures and the patient recovered after prolonged antimicrobial therapy.

Immunocompetent Host

CSD

Although most immunocompetent patients with CSD manifest with regional lymphadenopathy, an array of clinical abnormalities can develop, including Parinaud’s oculoglandular syndrome, encephalopathy, myelitis, peripheral neuropathy, neuroretinitis, granulomatous hepatitis, granulomatous splenitis, erythema nodosum, and osteolytic bone lesions. The initial clinical manifestation of CSD, the primary inoculation lesion, arises in more than 90% of patients, typically 3 to 10 days after the cutaneous inoculation of the organism by a cat scratch or bite. The primary inoculation lesion usually evolves through vesicular, erythematous, and papular (often with a covering crust) stages. In general, the primary inoculation lesion persists for about 1 to 3 weeks. Approximately 2 weeks (range, 3 to 50 days) after inoculation, patients develop adenopathy, the hallmark finding of CSD. Thus, when most patients present with regional lymphadenopathy, the primary inoculation lesion can still be observed, typically appearing at this stage as a crusted, erythematous papule.
More than 90% of patients with CSD present with regional lymphadenopathy, with the location of the adenopathy dependent on the site of the cat scratch or bite. Most commonly, the adenopathy develops in the axilla, cervical, or submandibular regions. Less commonly, the adenopathy may develop at epitrochlear, inguinal, femoral, or supraclavicular sites. Initially, the nodes are generally firm, tender, and 1 to 5 cm in diameter; overlying erythema may develop. About 85% of patients will have solitary lymphadenopathy, but some patients will have several abnormally swollen nodes in the same region. Less than 2% of patients will develop noncontiguous bilateral lymphadenopathy. Although generalized lymphadenopathy has been reported in a patient with CSD, this finding did not appear in a review of more than 1200 patients with CSD, and thus probably rarely occurs.

Although most patients have gradual resolution of their lymphadenopathy within several months, up to 20% experience prolonged adenopathy lasting longer than 6 months, even for as long as 12 to 24 months in some instances. Various series have reported widely variable rates of lymph node suppuration (depending on the severity of the lymph node enlargement), ranging from 12% to 48%. At the time patients present with lymphadenopathy, approximately one third will have a history of fever greater than 38.3°C, with the fever generally lasting at least 1 to 2 weeks. Similarly, approximately one third of patients complain of fatigue, but this often persists for longer periods.

About 2% to 3% of patients with CSD will develop Parinaud's oculoglandular syndrome, a disorder characterized by unilateral conjunctivitis and regional lymphadenitis. In 1985, Wear et al identified the CSD bacillus from conjunctival lesions in 9 of 24 patients with Parinaud's oculoglandular syndrome, thus adding the CSD bacillus to the list of other infectious agents known to cause this disorder. In patients with Parinaud's oculoglandular syndrome, the organism is inoculated on either the conjunctiva or eyelid via a scratch, lick, or bite; often the patient introduces the organisms into the eye by their own hand. Within several weeks after inoculation the patient develops preauricular adenopathy associated with either a nonpurulent conjunctivitis, a soft ocular granuloma, or both. Most patients do not appear seriously ill, and the abnormal ocular findings resolve within several months without residual ocular damage.

CSD-associated neurologic involvement probably occurs in at least 2% of patients with CSD, and can manifest as encephalitis, seizures, myelitis, peripheral neuropathy, or retinitis. In 1986, Lewis and Tucker reviewed the findings from 38 patients with CSD and neurologic involvement. Among these 38 patients, 34 had encephalopathy and 4 had predominantly myelopathic involvement. Patients with encephalopathy developed headache and mental status changes, typically 2 to 3 weeks after the onset of CSD; most (27 of the 34) had seizures at some point in their neurologic illness. Two of the 34 patients with encephalopathy had residual neurologic sequelae, 1 with a residual right hemiparesis and 1 with a complex partial seizure disorder that required chronic anticonvulsant drug therapy; both of these patients had focal abnormalities on computed tomographic scan at the time of their initial presentation. The four patients with myelitis had spinal cord or root involvement and presented with findings that included extremity weakness, sensory loss, reflex changes, and sphincter dysfunction; two of these four patients had a significant residual neurologic deficit.

In a more recent review published in 1991, Carithers and Margileth reported their findings in 61 patients with encephalopathy—the largest series of CSD-associated encephalopathy. The patients typically had an abrupt onset of neurologic symptoms approximately 1 to 6 weeks (range, 2 days to 2 months) after
their initial manifestation of CSD (usually regional lymphadenopathy). Existing data, however, do not suggest that patients with regional adenopathy in the neck region more frequently develop neurologic complications. Among the 61 patients described by Carithers and Margileth,21 46% had convulsions, 40% had combative behavior, 50% had a temperature greater than 38.2°C, and 26% had a temperature greater than 39°C. Laboratory studies, including CSF analysis and computed tomography of the brain, produced inconsistent and nondiagnostic results. Within 1 year, all 61 patients recovered, and most recovered within the first 12 weeks; none had permanent neurologic sequelae.

Carithers and Margileth22 also described 15 patients with neurologic abnormalities other than encephalopathy. These abnormalities included transient facial nerve paresis (Bell’s palsy) and transient peripheral neuropathy. In addition, they described 10 patients with neuroretinitis, each of whom suddenly developed unilateral blindness associated with regional lymphadenopathy and a cat scratch. These patients had gradual recovery of their vision during a 4- to 12-month period, with a return to normal (20/20) or almost normal vision (20/50). Wong et al.70 describe a woman who developed culture-proven bacteremia with *B. henselae* and acute loss of vision due to stellate neuroretinitis 2 months after acquiring a kitten. Her husband experienced similar symptoms and positive blood cultures 1 week later. *Bartonella henselae* also was isolated from the blood of the couple’s kitten.70

Several reports have described patients with CSD who had involvement of visceral organs.30,54,66 These reports describe patients with a 1- to 3-week history of fever with or without peripheral adenopathy. Dunn and colleagues recently described seven children with hepatosplenic CSD who presented with marked abdominal pain.34 The involvement of abdominal visceral organs is usually first suspected when either an abdominal ultrasound or computed tomography scan shows multiple defects in the liver (and sometimes the spleen). The lesions appear as round, oval, or irregular, typically ranging in size from 3 mm to 3 cm69. On noncontrast computed tomography scan, the lesions appear hypodense compared with normal parenchyma, but with contrast they may change to isodense or even slightly enhance. After several months, the lesions calcify or resolve. Patients with visceral organ involvement often have a self-limited clinical course.

In the Carithers review of 1200 patients, 5 patients with CSD developed erythema nodosum; these patients developed fever and subcutaneous nodules (on their shins) 1 to 6 weeks after the onset of regional lymphadenopathy, and all nodules resolved without sequelae.17 Several reports have also described a few CSD patients with osteolytic lesions.17,18,73 From the limited available data, it appears that these patients have an absence of several findings typically associated with osteomyelitis, namely fever and leukocytosis. One patient presented with a soft-tissue mass suggestive of a soft-tissue sarcoma.73

### Trench Fever

Most of the clinical descriptions of trench fever have been generated from the epidemics in World War I and World War II.57 Based on clinical observations, the incubation period appears to range from 3 to 38 days.57 In controlled studies, however, a more narrow range of 5 to 20 days was observed in human volunteers inoculated with *B. quintana*-infected louse feces.66 Four major fever patterns have been described with classic trench fever: (1) a single febrile episode with no subsequent fever; (2) a single febrile period lasting 4 to 5 days; (3) three to eight recurrent febrile episodes, each lasting 4 to 5 days; and (4) persistent fever
lasting 2 to 6 weeks. Among these four fever patterns, the episodic pattern occurs most frequently. On rare occasions, patients may develop infection without fever. Clinical signs and symptoms associated with these febrile episodes have varied and include malaise, myalgias, arthralgias, headache, conjunctivitis, retro-orbital pain, bone pain (particularly the shins), splenomegaly, and a transient macular rash.

**Bacteremia and Endocarditis**

Among the 10 patients involved in the cluster of *B. quintana* bacteremia from Seattle, 3 had weight loss that exceeded 20 lb, 7 had a temperature greater than 38.5°C, 1 had hypothermia (35.5°C), and two had splenomegaly. One patient initially presented with fever, anemia, cardiomegaly, a 3/6 systolic murmur, and a 3/6 diastolic murmur; echocardiography showed an aortic valve vegetation, and the patient subsequently underwent cardiac surgery with replacement of his aortic valve. A second patient developed signs and symptoms suggestive of endocarditis 9 months after his initial *B. quintana* bacteremia. Although echocardiography showed evidence of endocarditis and a computed tomography showed multiple splenic lesions, *B. quintana* was not isolated at this later time. One of the 10 patients died, but of causes probably unrelated to the *B. quintana* bacteremia.

In a report from France, three patients with *B. quintana* bacteremia developed endocarditis. All three patients presented with fever and two of three had significant weight loss (12 and 15 kg, respectively). Routine laboratory findings included thrombocytopenia (two patients), elevated erythrocyte sedimentation rate (two patients), and abnormal liver function tests (one patient). Cardiac valvular abnormalities involved the mitral valve (one patient), aortic valve (one patient), and both mitral and aortic valve (one patient); in all cases, the patient underwent cardiac surgery and had valve replacement.

A multicenter international study from France, England, Canada, and South Africa evaluated patients with blood culture-negative endocarditis and found 22 patients with *Bartonella* endocarditis, including 5 with *B. quintana* infection, 4 with *B. henselae*, and 13 with an undetermined *Bartonella* species. Among these 22 patients, 19 required valvular surgery and 6 died. Several other recent reports have described *Bartonella* endocarditis in immunocompetent hosts.

**EPIDEMIOLOGY AND PREVENTION**

**Immunocompromised Host**

Limited data exist regarding the incidence of *Bartonella* infections in immunocompromised patients, in part because systematic reporting of BA and bacillary peliosis hepatitis does not occur. Moreover, these disorders are probably underrecognized and underdiagnosed. Nevertheless, based on available reports it appears that *Bartonella* infections in immunocompromised patients are uncommon, but not rare.

Many of the initial BA reports noted prior cat contact among these patients. Subsequently, risk factors for developing BA were evaluated carefully in a case control study of 48 patients with BA or bacillary peliosis hepatitis. Exposure to a cat and, more specifically, receiving a cat scratch or bite, were significantly associated with the development of BA or bacillary peliosis. To further investigate this association between traumatic cat exposure and development of BA in
the pet owner, Koehler et al. identified four BA patients who still had the cats with which they had contact at the time they developed BA. Biopsy specimens were available for three of the patients, and the species causing BA was found to be *B. henselae*, not *B. quintana*, for each patient. Surprisingly, blood cultures of all seven of the cats that belonged to these four patients grew *B. henselae*, some with more than 1000 colony-forming units per milliliter of blood. Pet and pound cats in the San Francisco Bay area were then cultured, and 25 (41%) of 61 of the cats had *B. henselae* bacteremia. Most likely, a large number of cats are infected in other regions of the United States similar to the San Francisco Bay area. Childs et al. found that 89 (15%) of 592 banked cat sera from Baltimore had antibodies to *B. henselae*, with the highest seroprevalence (44%) in feral cats. In addition, Koehler et al. isolated *B. henselae* from some of the fleas combed from bacteremic cats, implicating the cat flea as a potential vector for *B. henselae* transmission (from cat to cat or from cat to human).

Preventing *B. henselae* infection is important for immunocompromised patients, many of whom are dependent on their pet cats as an important source of companionship. Screening cats by culture or for antibodies is not currently recommended because culture is difficult and cats can be intermittently bacteremic with *B. henselae*. One recent study found that absence of antibodies to *B. henselae* may be useful in predicting the absence of bacteremia in cats, but conversely, the presence of antibodies was not predictive of bacteremia. The very small potential risk of contracting *Bartonella* infection appears to be outweighed substantially by the benefit of cat ownership. It is therefore not recommended that immunocompromised individuals give up their pet cats, but that they make their medical caregivers aware that they own a cat, especially if they develop fever or cutaneous lesions. Immunocompromised individuals can reduce their exposure to feline-associated *B. henselae* by avoiding rough play that is likely to result in scratches, washing scratches and bites immediately with soap and water, and controlling cat flea infestation. Because younger cats are more likely to be bacteremic and to scratch, an immunocompromised person acquiring a pet cat should consider choosing an adult cat rather than a kitten. If an economical and reliable serologic test becomes available in the future, HIV-infected patients should consider choosing a seronegative cat.

One third of the patients in the epidemiologic study by Tappero et al. had no cat contact, and because *B. henselae* or *B. quintana* can cause BA, the reservoir and vector may be different for these two species. Although it appears that cats do not transmit *B. quintana* to humans, the source of BA caused by *B. quintana* remains unknown, and at present, the only recommendation for preventing infection with this species is to avoid contact with lice, a known vector of *B. quintana*.

**Immunocompetent Host**

Using a national database, Jackson and colleagues estimated an incidence of approximately 10 cases of CSD (hospitalized and ambulatory) per 100,000 persons per year in the United States, with a disproportionate percentage of males affected. Although most early studies suggested that CSD predominantly involved children, two recent large studies have shown that about 40% of CSD cases occur in persons older than 20 years of age. The disease appears to peak in the fall and early winter, perhaps related to the breeding patterns of cats and fleas. Available data suggest that CSD occurs across a broad geographic distribution, including most of North America. Zangwill and col-
leagues performed a detailed epidemiologic study in Connecticut and identified three risk factors among patients with CSD: (1) ownership of a pet kitten 12 months old or younger; (2) a scratch or bite from a kitten; and (3) ownership of at least one kitten with fleas. As noted previously, Koehler's study provided further evidence that cat fleas may play a significant role in the transmission of B. henselae. Moreover, a subsequent study has shown that cat fleas readily transmit B. henselae to cats.

Given the enormous number of North American households with cats or kittens, preventing CSD is an onerous task. Nevertheless, similar to the recommendations listed, cat owners should attempt to minimize the likelihood of B. henselae transmission by avoiding activities that may lead to a bite or scratch. Based on available data, cat owners should make a special effort to avoid bites or scratches from kittens.

Historically, trench fever has typically occurred in the setting of crowded, unsanitary conditions, such as in the trenches of the Western front during World War I and World War II. In the two recent reports of modern day B. quintana bacteremia that involved patients from Seattle and France, homelessness and alcoholism were identified as the major risk factors associated with B. quintana infection. Presumably, these conditions may have led to poor sanitary conditions that favored infestation with a vector, such as lice. Close person-to-person contact among individuals infested with a competent vector best explains these clusters of infections, but a clearly identified vector was not implicated in either of these retrospective studies. Although lice are the only known vector for B. quintana, other possible vectors, such as fleas, scabies, or ticks, could theoretically transmit B. quintana. Because the vector for modern day trench fever has not been established, no clear recommendations can be made regarding prevention of this infection in immunocompetent hosts. Identifying the vectors would take on increased importance if more cases of modern day trench fever are reported.

As a follow-up to the Seattle outbreak, patients seen at a downtown Seattle indigent clinic underwent serologic testing; 20% of patients had B. quintana antibody titers of at least 1:64 compared with only 2% of blood donors. Similarly, Brouqui and co-workers performed a serologic study in France and found 16% of hospitalized homeless patients had positive antibodies to B. quintana whereas none of 250 serum samples from blood donors tested positive. Last, Comer and colleagues conducted a serologic study that involved 630 inner-city individuals from Baltimore and found more than 37% showed reactivity to Bartonella antigens. The investigators used three Bartonella antigens for testing; B. elizabethae, B. henselae, and B. quintana. Reactive antibodies were associated with injection drug use and HIV seronegative status.

**DIAGNOSTIC TESTS**

**Histopathology**

To distinguish between BA and KS, all immunocompromised patients with new vascular-appearing cutaneous lesions should have a lesion biopsied. In a patient with biopsy-proven KS, a new vascular proliferative lesion that has a different appearance compared with the patient's other KS lesions also should be biopsied. The biopsy specimen should be examined by hematoxylin and eosin staining, which differentiates BA from KS, and by Warthin-Starry staining, a stain that identifies the bacillary organisms in BA lesions. Bacillary angiomatosis of skin and other organs is characterized by a lobular proliferation of
small capillaries, each of which is lined with endothelial cells. The endothelial cells in BA lesions are protuberant, unlike the endothelial cells of KS that usually appear spindle-shaped and form slit-like spaces.\textsuperscript{52} The \textit{Bartonella} bacilli are usually located in the extracellular matrix adjacent to the proliferating endothelial cells, and are rarely intracellular. The inflammatory infiltrate usually includes both lymphocytes and neutrophils, with focal areas of necrosis.\textsuperscript{53}

Microscopic examination of the lesions of bacillary peliosis hepatis reveals histopathologic characteristics distinct from those of BA. Lesions of bacillary peliosis show dilated capillaries and larger, even macroscopically visualized cystic, blood-filled spaces scattered throughout the hepatic or splenic parenchyma. The lining of these cystic spaces is often thin and devoid of the protuberant endothelial cells observed in BA lesions.\textsuperscript{56} Clumps of bacilli are seen adjacent to the peliotic spaces, best visualized with Warthin-Starry silver deposition staining as with BA lesions.

Findings on lymph node biopsy specimens from immunocompetent patients with CSD depend on the stage of the infection.\textsuperscript{49} Early in the course, specimens show lymphoid hyperplasia, arteriolar proliferation, and reticulum cell hyperplasia. Subsequently, granulomas appear, often with central necrosis; at this stage, multinucleate giant cells may become evident. Late in the disease, the specimen characteristically shows multiple stellate microabscesses. Similar to BA, routinely stained specimens generally do not show microorganisms. Warthin-Starry stains, however, show clumps of pleomorphic bacilli, primarily in the walls of blood vessels, in macrophages lining the sinuses, and in microabscesses. Biopsy samples from either liver or spleen typically show multiple granulomas and stellate abscesses;\textsuperscript{49} in some instances, organisms have been observed with Warthin-Starry staining.

**Culture from Clinical Specimens**

Species of the \textit{Bartonella} genus are small, gram-negative rods that are extremely fastidious and require special culture conditions for isolation.\textsuperscript{41} The isolation from blood of cats\textsuperscript{115,43} and humans\textsuperscript{88} is optimally accomplished by use of pediatric or adult Isolator tubes (Wampole, Cranbury, NJ) or EDTA blood tubes. Recovery of \textit{Bartonella} species can be achieved by plating samples onto either heart infusion agar supplemented with 5% rabbit blood or chocolate agar, but without added antimicrobials.\textsuperscript{57,66} The agar plates must be fresh, and after inoculation, should be incubated for at least 21 days in 5% CO\textsubscript{2} at 35 to 37° C. In addition, acridine orange staining, when performed 8 days after incubation, has been used to identify growth of \textit{B. quintana} in BACTEC bottles (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD), with the organisms appearing as pale orange amorphous clumps.\textsuperscript{50} Once identified, the organisms can then be recovered by subculturing onto chocolate agar. The sensitivity of the acridine orange staining technique decreases in patients who have received recent or concurrent antimicrobial therapy.

Recovery of \textit{Bartonella} species from BA and CSD lesions remains very difficult. \textit{B. henselae} and \textit{B. quintana} have been isolated from cutaneous BA lesions by direct plating of tissue homogenate onto agar and by cocultivation with a bovine endothelial cell monolayer.\textsuperscript{57} Dolan et al\textsuperscript{114} isolated \textit{B. henselae} from CSD lymph nodes of two patients using direct plating of tissue homogenate onto both chocolate agar and CDC anaerobic blood agar; colonies were detected after 13 days for one patient and 33 days for the other.
Serology

Using an indirect immunofluorescence assay (IFA) developed at the CDC, Tappero et al. found *Bartonella* antibodies in seven of seven patients with biopsy-confirmed BA, but in none of seven HIV-infected patients without BA. Interestingly, banked sera from three of the seven BA patients showed presence of *Bartonella* antibodies for up to 7 years before the development of BA. Moreover, the three patients with banked sera had a fourfold rise in *Bartonella* antibody titers prior to the appearance of clinically evident lesions, and another patient had elevated antibody titers that predicted a relapse of infection following 4 months of antimicrobial therapy. As noted previously, one study has shown an increased incidence in serum and CSF *Bartonella* antibodies among HIV-infected patients with neuropsychiatric decline.

Using an indirect IFA to detect antibodies to *B. henselae*, Regnery et al. showed that 36 (88%) of 41 persons with CSD had an antibody titer of at least 1:64. Using the same cut off, only 6 (6%) of 107 healthy controls had a positive test. False-positive results (antibody titer of at least 1:64) were observed in 2 of 10 patients with brucellosis and in 1 of 3 patients with Lyme disease. Eight patients with *B. henselae* antibody titers of at least 1:64 also had low titers against *B. quintana* (less than or equal to 1:32). Subsequently, Dalton and co-workers reported that among 91 persons who had an illness that met a strict clinical definition of CSD, 87 (95%) had antibody titers of at least 1:64 to either *B. henselae* or *B. quintana*. Barka et al. used an enzyme immunoassay (EIA) method to detect antibodies to *B. henselae*, and found 38 (95%) of 40 patients with confirmed CSD had elevated (cut off of 12 units [0.250 OD<sub>450</sub>]) antibody titers to either IgG, IgM, or IgA. They did not find positive antibody responses to *B. henselae* when they tested 92 serum samples from patients who had documented high antibody titers to other infectious agents, such as *Brucella abortus*, *Rickettsia typhi*, *Rickettsia rickettsii*, *A. felis*, *Borrelia burgdorferi*, *Yersinia pestis*, *Francisella tularensis*, *Chlamydia trachomatis*, cytomegalovirus, and rubella.

In the 1970s, Hollingdale et al. compared an EIA assay with counterimmunoelectrophoresis (CIE) to diagnose trench fever. They found all patients with primary or relapsed trench fever to have elevated antibody titers (1:20–1:640) using EIA and positive (one to three precipitin lines) CIE tests. These authors, however, did observe significant cross-reactivity in patients infected with either *R. tsutsugamushi* or *R. typhi*. In the recent report from France that involved three patients also with *B. quintana* endocarditis, Drancourt et al. used a microimmunofluorescent test and found all three patients to have elevated *B. quintana* antibody titers, but all three patients also had elevated antibody titers to *Chlamydia pneumoniae*. In a separate study, La Scola and Raoult showed that more than 50% of patients with chronic *Coxiella burnetii* infection (chronic Q fever) had antibody tests positive for *B. henselae*.

Given the available serologic data, it appears that most patients with either BA, CSD, or trench fever will have positive *Bartonella* antibody titers. These serologic tests generally show relatively high specificity (to the genus level), with a few exceptions (as noted previously). Although one group has used IFA, enzyme immunoassay, and immunoblots in a murine model to distinguish between *B. quintana* and *B. henselae*, the ability to clearly differentiate one *Bartonella* species from another in serologic tests in humans has not been well established. Although serologic tests may take on an increasingly important role in the diagnosis of BA, CSD, and trench fever, their role at the present time has not been clearly defined. In the United States, three commercial laboratories offer *B. henselae* antibody testing: (1) Associated Regional University Pathologists
(Salt Lake City, Utah), (2) Specialty Laboratories, Inc. (Santa Monica, California), and (3) Microbiology Reference Laboratory (Cypress, California). The sensitivity and specificity of these commercial tests have not been compared in a peer-reviewed publication.

**PCR-Based DNA Detection of *Bartonella* Species**

PCR has been a very useful technique in demonstrating *Bartonella* DNA in clinical materials, especially because culturing and isolating these fastidious bacilli have remained extremely difficult. PCR detection techniques remain experimental, however, and are thus not routinely available. Relman et al. developed the first PCR primers specific for detection of *Bartonella* DNA. These primers amplify a 296 base pair fragment of the 16S ribosomal RNA gene (rDNA). Within this fragment there are four hypervariable nucleotides that can be used to distinguish the four *Bartonella* species that affect humans, but this technique requires sequencing the entire amplified segment. Koehler et al. used these 16S rDNA primers to show that the *Bartonella* species isolated from cutaneous BA lesions were *B. quintana* in some cases and *B. henselae* in others; this speciation was subsequently confirmed by a more specific test: DNA-DNA hybridization.

Bergmans et al. also used these 16S rDNA primers with blotting and hybridization to detect *Bartonella* DNA in 85 (96%) of 89 pus and lymph node specimens from CSD patients. They also designed PCR primers for *A. felis* DNA detection, but were unable to identify *A. felis* DNA in any of the same 89 clinical CSD specimens. Different primers were developed by Anderson et al. and used in conjunction with dot blot hybridization to detect *B. henselae* DNA in clinical specimens from CSD patients. Using this combination of techniques, they were able to demonstrate *B. henselae* DNA in 21 (84%) of 25 lymph node specimens from CSD patients. *B. quintana* DNA was not detected in any of the same specimens. Amplification of the citrate synthase gene (or a gene fragment), with subsequent sequencing or restriction fragment length polymorphism analysis, also has been used to distinguish among *Bartonella* species. These tests are not commercially available, but development of a widely available PCR-based test would facilitate evaluation of patients with suspected CSD or BA.

**Skin Tests**

Historically, a positive CSD cutaneous antigen test was part of the diagnostic criteria for CSD. The material used for this test is derived from lymph nodes obtained from patients with CSD. The CSD antigen material (0.1 cc) is injected intradermally, with a reaction of at least 5 mm of induration at 72 hours considered a positive test. Most studies suggest that the CSD antigen is positive in approximately 90% of patients with CSD. This test, however, may be falsely negative in patients with an illness less than 4 weeks in duration, and it does not differentiate present from past infection. In recent years, investigators have analyzed CSD antigen material and have detected *B. henselae* DNA (but not *A. felis* DNA). Given the potential for transmission of infectious pathogens with this test, most clinicians currently depend on other methods to diagnose CSD.
THERAPY

Immunocompromised Host

Antimicrobial treatment for immunocompromised individuals with *Bartonella* infection is beneficial and clearly indicated. Although treatment of BA and bacillary peliosis has not been studied systematically, experience with treatment of approximately 50 patients at San Francisco General Hospital and summarized from numerous case reports demonstrates that lesions and symptoms respond rapidly to erythromycin or doxycycline therapy. Other antimicrobials used to successfully treat BA in immunocompromised patients include tetracycline, minocycline, and azithromycin. Rifampin may have activity against *Bartonella* species in vivo, but because efficacy has not been established with this drug alone, use of rifampin is recommended as a second drug in combination with either erythromycin or doxycycline for severely ill patients.

A case report details treatment of a pregnant patient with BA using ceftizoxime, but there is inadequate experience with the third-generation cephalosporins, trimethoprim-sulfamethoxazole, or quinolone antimicrobials to recommend their use at present. Our experience and numerous case reports have clearly shown that BA lesions do not adequately respond to the penicillins and first-generation cephalosporins. In addition, the published in vitro susceptibilities have not correlated completely with the in vivo response of *Bartonella*, particularly with regard to the penicillins and first-generation cephalosporins.

Clinicians should advise immunocompromised patients with BA or bacillary peliosis that they may develop a Jarisch-Herxheimer reaction after the first several doses of antimicrobials. Patients with only cutaneous BA should receive at least 2 months of antimicrobial therapy, and patients with either osteomyelitis or peliosis hepatitis should receive at least 4 months. Numerous published reports detail relapse of *Bartonella* infection in immunocompromised patients, in part because of the shorter duration of treatment used several years ago, but also probably because of the relapsing nature of *Bartonella* infections, even in immunocompetent individuals. If relapse occurs, the HIV-infected patient should receive life-long treatment with erythromycin or doxycycline.

Immunocompetent Host

In contrast to the excellent responses observed with HIV-infected patients who have BA, treatment responses of immunocompetent individuals with CSD have been disappointing. In a 24-month uncontrolled retrospective study, Margileth analyzed treatment responses in 202 patients with CSD who took at least 3 days of antimicrobial therapy. Among 18 antimicrobials used, only four (rifampin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole) appeared to provide any clinical benefit. Two other reports that involved a total of four patients suggested that intravenous gentamicin may shorten the course of CSD. A separate report described five adult patients who had rapid improvement in symptoms after receiving oral ciprofloxacin. Results of in vitro susceptibility testing often do not correlate with clinical outcome, and therefore should generally not be used to guide therapy.

Clear guidelines for the treatment of CSD do not exist, mainly because no randomized controlled trials have been performed. Given the lack of data showing a clear benefit of antimicrobial therapy in the treatment of patients with mild to moderate CSD, most authorities have recommended managing
these patients without antimicrobial therapy. Based on the limited available data (and considering the experience in immunocompromised hosts), if therapy is given to patients with mild to moderate disease, it would be reasonable to attempt a 10- to 14-day trial of oral therapy with either doxycycline or erythromycin. For patients with severe disease (including central nervous system disease), we recommend intravenous doxycycline with or without addition of rifampin. The reasons for the relatively poor response of patients with CSD compared with patients who have BA remain unclear, but may relate to differences in host inflammatory response or organism load.

Published data regarding antimicrobial therapy for trench fever have been sparse. In the recent Seattle outbreak, most patients received a β-lactam agent followed by either erythromycin or azithromycin, generally with satisfactory results (if given for at least 14 days); in this series of patients, patients did not receive therapy as part of a trial, and follow-up was limited. Most cases of endocarditis have required valve replacement despite intravenous antimicrobial therapy. Because of the sporadic nature of this illness, large controlled treatment trials will probably not be performed. The lack of clinical data, combined with unreliable in vitro susceptibility testing, make clear treatment guidelines unrealistic. Nonetheless, based on limited data and extrapolating from experience with HIV-infected patients, the authors recommend administering either erythromycin, azithromycin, doxycycline, or tetracycline for at least 14 days for patients with uncomplicated bacteremia. Patients with endocarditis should receive prolonged therapy (at least 4 to 6 months), and should be carefully followed for clinical deterioration that would require valve replacement. In addition, further research is needed to determine whether a bactericidal agent, such as a third-generation cephalosporin, is necessary for patients with uncomplicated bacteremia or endocarditis.

CONCLUSIONS AND FUTURE DIRECTIONS FOR BARTONELLA RESEARCH

The recent years have produced a tremendous advance in the understanding and appreciation of Bartonella-associated infections. It has become clear that Bartonella can infect immunocompromised and immunocompetent individuals, but distinct clinical manifestations develop in these two groups. Many questions, however, remain unanswered. For instance, does Bartonella have an even broader spectrum of manifestations that have not, as of yet, been appreciated? Why does antimicrobial therapy have a much greater impact on immunocompromised persons with Bartonella infections than those who are immunocompetent? Can large studies define effective antimicrobial therapy for patients with CSD, and will effective antimicrobial therapy require more than one agent? What is the vector for B. quintana in the United States? What is the natural history of patients seropositive for Bartonella? What is the extent of B. quintana infections in areas outside of Seattle, Baltimore, or France? These and many other questions will likely surface in the upcoming years and will require well-designed research studies to answer.

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