Use of Retinal Biopsy to Diagnose *Bartonella* (Formerly *Rochalimaea*) *henselae* Retinitis in an HIV-Infected Patient

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A patient with the acquired immunodeficiency syndrome developed bilateral retinitis due to a *Bartonella* (formerly *Rochalimaea*) *henselae* infection. A retinal biopsy was performed when severe and progressive retinal infection failed to respond to empirical treatment for cytomegalovirus and *Toxoplasma gondii*. The biopsy specimen was stained with routine histopathological stains and the Steiner silver stain. Ribosomal DNA was extracted from formalin-fixed, paraffin-embedded retinal tissue and amplified with the polymerase chain reaction assay, using *Bartonella*-specific primers. The amplified DNA fragment was cloned and sequenced. Staining with hematoxylin-eosin revealed tufts of proliferating vascular endothelium with numerous fusiform-appearing cells, consistent with a diagnosis of bacillary angiomatosis. A Steiner silver stain revealed numerous small bacilli in the biopsy specimen. Amplification of DNA extracted from the tissue produced a fragment of 16S ribosomal DNA of the expected size; sequencing of the DNA fragment revealed that the infection was caused by *B henselae*. The retinal infection was treated with minocycline, doxycycline, and ciprofloxacin with improvement in visual acuity in the ensuing 12 weeks. To our knowledge, this is the first human immunodeficiency virus–infected patient with retinitis due to *B henselae* who was diagnosed by the identification of silver-staining bacilli and amplification and sequencing of *B henselae* with a polymerase chain reaction assay using a biopsy specimen of retinal tissue. Retinal biopsy is indicated, despite its potential for serious complications, in patients with acquired immunodeficiency syndrome who have a progressive, sight-threatening retinitis that is undiagnosed and unresponsive to therapy.

Infectious retinitis is a common complication of human immunodeficiency virus (HIV) infection that is most often due to cytomegalovirus (CMV) but can also be due to less common pathogens such as *Toxoplasma gondii* and *Treponema pallidum*. Recently, *Bartonella* (formerly *Rochalimaea*) *henselae*, the etiologic agent of cat-scratch disease, was recognized as another uncommon cause of retinitis in patients with HIV infection. Retinitis due to *B henselae* is difficult to diagnose clinically because the ophthalmologic findings are often indistinguishable from those of other infections and results of antibody tests can be nondiagnostic.

We recently cared for a patient with the acquired immunodeficiency syndrome (AIDS) who developed a bilateral, progressive retinitis that was diagnosed as a *B henselae* infection by identifying the bacteria in a retinal biopsy specimen with the Steiner variation of the Warthin-Starry stain and the polymerase chain reaction assay (PCR). Since we are unaware of previous reports of the usefulness of retinal biopsy in diagnosing *B henselae* infection, we are documenting this case.

A 33-year-old man with an 8-year history of HIV infection was referred to an ophthalmologist in October 1995 with blurred
vision. His history revealed *Pneumocystis carinii* and CMV pneumonias within the past 2 years and contact with a pet cat. The *Pneumocystis* infection that was diagnosed by finding *P carinii* in bronchoscopic, blood, and urine specimens, and by demonstrating elevated IgM and IgG antibody titers of 1:320 and 1:1024. This infection improved with parenteral ganciclovir therapy. Findings from the ophthalmologic examination in October 1995 revealed a visual acuity of 20/20 OU, normal intraocular pressures, and no anterior segment inflammation. Funduscopic examination revealed a focal area of hemorrhagic retinal necrosis in the left eye with multiple midperipheral intraretinal hemorrhages. There were several cotton-wool spots noted in the right eye without other evidence of retinal inflammation. A macular star was not noted in either eye. Results of the VDRL, toxoplasma, and histoplasma antibody and the cryptococcal antigen tests were negative. The CD4 count was 0.04 × 10⁹/L (39/mm³). A presumptive diagnosis of CMV retinitis was made and the patient was treated with parenteral ganciclovir. Despite treatment, the retinal lesion in the left eye enlarged and the patient’s vision worsened to only hand motion. Because of increasing anterior segment inflammation, worsening retinitis, vitritis, and an inferior exudative detachment, a diagnostic vitrectomy was performed in the left eye.

**HISTOLOGIC FINDINGS**

Findings from histologic examination of the vitreous sample revealed inflammatory cells. Results of cultures taken of the vitreous for herpes, CMV, fungi, aerobic and anaerobic bacteria, and mycobacteria were negative. Since the patient’s vision had deteriorated despite adequate therapy for CMV retinitis, he was treated empirically with pyrimethamine and sulfadiazine and then pyrimethamine and clindamycin for the possibility of toxoplasmosis. The left eye lesion improved while taking the pyrimethamine and clindamycin regimen. However, this regimen provoked headache and abdominal discomfort necessitating a change to the combination drug trimethoprim-sulfamethoxazole. This regimen was also discontinued because of side effects. Two months later, a new lesion was noted in the contralateral eye just temporal to the macula (**Figure 1**). An additional lesion was present in the peripheral retina. Because the clinical appearance again suggested toxoplasmosis, the patient was treated once more with pyrimethamine and clindamycin. Findings from repeated *Toxoplasma* titers remained negative, and no definitive diagnosis was made. The patient’s vision continued to worsen, and the lesion temporal to the macula enlarged despite treatment. Because of the clinical progression and lack of a diagnosis, a pars plana vitrectomy, lensectomy, and retinal biopsy were performed in September 1996. The biopsy tissue was fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin as well as the Steiner variation of the Warthin-Starry stain. The stained slides revealed multiple tufts of proliferating vascular endothelial cells with fibroblasts and fusiform-appearing cells consistent with a diagnosis of bacillary angiomatosis (**Figure 2**). Because this entity has clearly been associated with the *Bartonella* species, an additional stain was done using the Steiner variation of the Warthin-Starry stain, which revealed clusters of minute bacteria compatible with *B henselae*.

The PCR for *Bartonella* DNA was performed as follows. Template DNA was extracted from the paraffin-embedded biopsy tissue of the retina and normal human skin (negative tissue extraction control) and an agar-grown single colony of *B henselae*-type strain (positive DNA control). Special precautions were taken to prevent contamination with exogenous DNA during extraction of DNA and PCR amplification. Amplification of the *Bartonella* 16S ribosomal RNA gene (*rDNA*) was performed using *Bartonella*-specific oligonucleotide primers p24E and p12B (from University of California, San Francisco Biomolecular Resource Center) under conditions previously described. Human β-globin primers (Operon, Berkeley, Calif) also were used to en-
ensure absence of inhibitory substances in the PCR mixtures containing DNA extracted from the retinal lesion and normal human skin. A negative PCR control (containing all the reagents and water in place of the template DNA) was included with each PCR group. The amplified 16S rDNA fragment was cloned and sequenced as previously described. These sequences included with each PCR group. The amplified fragments were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. Lanes 1 and 2 show DNA size standards. Lane 2 is a negative PCR control with deuterium oxide (heavy water) in place of a DNA template. Lane 3 shows amplification of a 298-base pair (bp) product (arrowhead) with Bartonella (formerly Rochalimaea) henselae-type strain DNA, using primers p24E and p12B. Lane 4 shows amplification of an identically sized product with p24E/p12B and DNA extracted from brain lesion biopsy tissue. Lanes 5 and 6 show amplification of an approximately 268-bp band using human β-globin primers with brain tissue DNA template (lane 5) or DNA template from simultaneously extracted normal skin tissue (lane 6). Lane 7 shows no product using primers p24E/p12B and control (normal human skin) DNA template. The expected 298-bp product was amplified using Bartonella-specific primers with DNA extracted from a type strain of B. henselae and retinal lesion biopsy tissue. Bartonella-specific primers did not amplify any product using DNA extracted from normal skin biopsy tissue. Human β-globin primers amplified a 268-bp product from the DNA template of the retinal lesion biopsy tissue and human skin tissue, indicating absence of inhibitory substances.

Figure 3. Polymerase chain reaction assay (PCR)–amplified 16S ribosomal rDNA fragments from tissue and bacterial DNA extracts. DNA was extracted simultaneously from formalin-fixed, paraffin-embedded tissue from the retinal biopsy specimen and from control tissue and was amplified by PCR. The amplified fragments were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. Lanes 1 and 8 show DNA size standards. Lane 2 is a negative PCR control with deuterium oxide (heavy water) in place of a DNA template. Lane 3 shows amplification of a 298-base pair (bp) product (arrowhead) with Bartonella (formerly Rochalimaea) henselae–type strain DNA, using primers p24E and p12B. Lane 4 shows amplification of an identically sized product with p24E/p12B and DNA extracted from brain lesion biopsy tissue. Lanes 5 and 6 show amplification of an approximately 268-bp band using human β-globin primers with brain tissue DNA template (lane 5) or DNA template from simultaneously extracted normal skin tissue (lane 6). Lane 7 shows no product using primers p24E/p12B and control (normal human skin) DNA template. The expected 298-bp product was amplified using Bartonella-specific primers with DNA extracted from a type strain of B. henselae and retinal lesion biopsy tissue. Bartonella-specific primers did not amplify any product using DNA extracted from normal skin biopsy tissue. Human β-globin primers amplified a 268-bp product from the DNA template of the retinal lesion biopsy tissue and human skin tissue, indicating absence of inhibitory substances.

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Results of serum antibody titers that were performed at a commercial laboratory were negative, but a later specimen sent to the Centers for Disease Control and Prevention in Atlanta, Ga, showed titers to B. henselae and Rochalimaea quintana of 1:64 and 1:128, respectively. Titers of 1:64 are considered evidence of infection with B. henselae and patients with cat-scratch disease typically have high titers to R. quintana because of cross-reactivity.6

The patient was treated with a 12-week course of 200 mg of minocycline or doxycycline daily and 500 mg of ciprofloxacin twice daily for 6 of these weeks with resolution of the retinal lesion and improvement in his visual acuity. He also had a secondary intraocular lens placed in the posterior chamber of the right eye. Follow-up examinations in 1997 have revealed quiescence of the intraocular inflammation and resolution of the retinitis. Macular striae and epithelial change limit the visual acuity in both eyes. There is a healing scar temporal to his macula present in the right eye after treatment (Figure 4). During this period, the patient received lamivudine, stavudine, and saquinavir for his HIV infection. On this regimen, he gained 6.75 kg and his CD4 cell count increased to $0.06 \times 10^9/L$.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Polymerase chain reaction assay (PCR)–amplified 16S ribosomal rDNA fragments from tissue and bacterial DNA extracts. DNA was extracted simultaneously from formalin-fixed, paraffin-embedded tissue from the retinal biopsy specimen and from control tissue and was amplified by PCR. The amplified fragments were separated by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. Lanes 1 and 8 show DNA size standards. Lane 2 is a negative PCR control with deuterium oxide (heavy water) in place of a DNA template. Lane 3 shows amplification of a 298-base pair (bp) product (arrowhead) with Bartonella (formerly Rochalimaea) henselae–type strain DNA, using primers p24E and p12B. Lane 4 shows amplification of an identically sized product with p24E/p12B and DNA extracted from brain lesion biopsy tissue. Lanes 5 and 6 show amplification of an approximately 268-bp band using human β-globin primers with brain tissue DNA template (lane 5) or DNA template from simultaneously extracted normal skin tissue (lane 6). Lane 7 shows no product using primers p24E/p12B and control (normal human skin) DNA template. The expected 298-bp product was amplified using Bartonella-specific primers with DNA extracted from a type strain of B. henselae and retinal lesion biopsy tissue. Bartonella-specific primers did not amplify any product using DNA extracted from normal skin biopsy tissue. Human β-globin primers amplified a 268-bp product from the DNA template of the retinal lesion biopsy tissue and human skin tissue, indicating absence of inhibitory substances.

This case illustrates the value of retinal biopsy in the diagnosis of infectious retinitis due to B. henselae in patients with AIDS in whom the diagnosis is not established by findings from ophthalmologic examination and serum antibody testing. Our patient’s ophthalmologic examination findings revealed losses of visual acuity and multifocal whitish retinal lesions consistent with B. henselae infection; but, since these ophthalmologic findings are also present in other infections, they are not etiologically specific.5,6 The indirect fluorescent antibody test can also establish the diagnosis of B. henselae infection, but this test too can be non-diagnostic as the sensitivity is 84% and the specificity is 96%.5 In our patient, the 2 serum antibody results showed an absence of antibody at the time of the retinal biopsy in September 1996 and the presence of antibody in the specimen tested by the Centers for Disease Control and Prevention in November. Whether these results are attributable to a seroconversion during this period or a falsely negative result in September is uncertain. Regardless of the cause for the differences in the test result, the retinal biopsy showed B. henselae as the etiologic cause for the retinitis, thus demonstrating the diagnostic value of biopsy in patients with AIDS who have an undiagnosed, progressively worsening retinopathy.

Retinal biopsy is a radical procedure fraught with complications (vitreous hemorrhage, retinal detachment, hypotony) that is recommended for patients like ours in whom a sight-threatening retinitis is not responding to therapy.13 The biopsy specimen demonstrated large numbers of silver-staining bacteria consistent with B. henselae using the Steiner variation of the Warthin-Starry stain. This finding is in agreement with results of previous investigations showing that this staining procedure detects B. henselae.14 Bartonella henselae DNA was also detected with the PCR. It is worth emphasizing that these results showed that very small biopsy samples are...
sufficient for microscopic detection and PCR identification of the pathogen. Although we did not obtain cultures from the retinal biopsy, \textit{B. henselae} can be cultured, albeit with difficulty, on artificial media such as chocolate agar, charcoal-yeast extract agar, and Isolator (Wampole Laboratories, Cranberry, NJ) and BACTEC (Becton Dickinson, Sparks, Md) blood culture systems.\textsuperscript{4}

The finding that treatment with minocycline, doxycycline, and ciprofloxacin improved the patient’s retinitis agrees with previous reports of therapeutic benefit with these antibiotics.\textsuperscript{6} Additional antibiotics that have been used to treat \textit{B. henselae} retinitis are rifampin, tetracycline, and erythromycin.\textsuperscript{4} Because eye infections due to \textit{B. henselae} respond to antibiotic treatment, a course of empiric therapy is a reasonable therapeutic choice in suspected but unproven cases.

In summary, infectious retinitis in a patient who has AIDS can be due to a number of causes. Although CMV is the most common cause, other causes such as \textit{B. henselae} are also important and these causes for retinitis are difficult to diagnose by clinical examination. The diagnostic problem is further complicated when the origin is not determined by laboratory testing. In these situations, a retinal biopsy, despite the potential for serious complications, is indicated to provide a diagnosis in these patients who have a progressive, sight-threatening retinitis.

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