Fatal Pancarditis Associated with Human Granulocytic Ehrlichiosis in a 44-Year-Old Man


From the Department of Laboratory Medicine and Pathology and the Department of Medicine, Division of Cardiology, Mayo Clinic, Rochester, Minnesota; Marshfield Clinic, Marshfield, Wisconsin; and the Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland

Human cases of infection with a granulocytotropic Ehrlichia species closely related to Ehrlichia equi are now being described with increasing frequency in the United States, especially in areas where Lyme disease is already endemic. We describe a case of fatal pancarditis during the course of human granulocytic ehrlichiosis (HGE) in a 44-year-old outdoor worker who was previously treated for presumptive Lyme disease. Serological and molecular diagnostic tests for Borrelia burgdorferi and Babesia microti infections were negative. Postmortem serum specimens were seroreactive for HGE, and molecular evidence of infection with the HGE agent was obtained. These findings suggest that carditis may be a manifestation of HGE, further complicating the differential diagnosis of tick-borne illness.

Recently, cases of ehrlichial infection due to an agent closely related to Ehrlichia equi have been reported in areas where Lyme disease is considered endemic [1]. In contrast to Ehrlichia chaffeensis, which infects mononuclear leukocytes, E. equi and its relatives infect neutrophils, and the illness caused by this organism has been termed human granulocytic ehrlichiosis (HGE). Detection of this organism along with Borrelia burgdorferi in deer ticks [2, 3] and the frequent detection of antibody specific to Ehrlichia in sera from patients with Lyme disease [2] suggest that human exposure to one or both organisms occurs via a common transmission cycle. Since diseases due to both organisms may present initially as a nonspecific febrile illness [4–7], they may be difficult to distinguish on clinical grounds. Herein, we describe a case of sudden cardiac death due to myocarditis during HGE that was initially mistaken for acute Lyme disease.

Case Report

A previously healthy 44-year-old male outdoor worker from Brainerd, Minnesota presented to his local physician on 7 July 1994 because of a 1-week history of fever, chills, generalized myalgias, and right temporomandibular joint pain. Fourteen days before presentation he had removed two deer ticks that had been attached to his skin for an unknown period. He had no other symptoms and denied any skin reactions at that time. He treated himself empirically for presumptive early Lyme disease with ampicillin, which was available at home, for 2 days; because there was no clinical improvement, he sought medical attention.

Physical examination was unremarkable except for the presence of a low-grade fever (temperature, 100.6°F). Results of examinations of the heart, lungs, and abdomen were reportedly normal, and lymphadenopathy, hepatosplenomegaly and skin rash were not noted. No laboratory, radiological, or electrocardiographic evaluations were performed at that time. There was no history of any significant medical illnesses. He smoked one and one-half packs of cigarettes per day for 20 years and had a family history of coronary artery occlusive disease. Cardiovascular, pulmonary, gastrointestinal, and neurological symptoms were not present. A presumptive diagnosis of acute Lyme disease was made, and empirical treatment with amoxicillin (500 mg q.i.d.) and probenecid (500 mg t.i.d.) was initiated; a 1-week follow-up visit was planned.

The 19th day after the tick bite, the patient died suddenly at home. He had complained of slight shortness of breath the day before his death, but there were no complaints of antecedent chest pain, palpitations, light-headedness, dizziness, orthopnea, or paroxysmal nocturnal dyspnea.

Methods

Serological Testing for Tick-Transmitted Agents

Postmortem serum specimens were evaluated initially for presumptive carditis due to B. burgdorferi [8]. Serum specimens were analyzed in accord with a two-tiered serodiagnostic approach in which a sensitive serological screening test is used to first identify seroreactivity to B. burgdorferi followed by immunoblotting for confirmation of reactivity determined by...
an ELISA [9, 10]. Serological tests were performed in two reference laboratories with extensive experience in serological evaluations for Lyme disease (laboratory 1, Mayo Clinic, Rochester, MN; laboratory 2, Marshfield Clinic, Marshfield, WI). Initial screening procedures for detection of levels of total immunoglobulin were performed by using two different ELISAs for detection of antibody to *B. burgdorferi* (laboratory 1, MardX Lyme ELA, Carlsbad, CA; laboratory 2, Gen Bio Lyme ELA, Gen Bio, San Diego). In laboratory 1, the sample was tested by western immunoblotting for IgG antibody (MardX Lyme western blotting). In both laboratories, an indirect immunofluorescent antibody screening test for IgM antibody to *B. burgdorferi* was also performed with use of a commercially available indirect immunofluorescent antibody substrate containing a low-passage isolate of strain B31 (Bion, Chicago). Serum dilutions of 1:32 and 1:64 were tested.

Serological evaluation was further supplemented in laboratory 1 with the Recombinant Immunodot assay (GenBio, San Diego); for detection of IgM and IgG antibodies to *B. burgdorferi* containing recombinant *B. burgdorferi* antigens ospC, flagellin, p39, and p83. For the serological diagnosis of HGE and *Babesia microti* infection, indirect fluorescent antibody testing for IgM antibody screening test for IgG antibody and western immunoblotting, immunoassays for IgG and IgM antibodies to *B. burgdorferi* were negative by means of a conventional ELISA for IgG antibody and western blotting, immunoassays for IgG and IgM antibodies to recombinant *B. burgdorferi* antigen, as well as a sensitive indirect immunofluorescent antibody IgM assay for early Lyme disease. Direct immunofluorescence for detection of *B. microti* infection was also negative, as were PCR studies of serum and clotted whole blood specimens for both *B. burgdorferi* and *B. microti* [11, 14]. However, titers of antibody to *E. equi* were significantly elevated at >1:256 according to indirect immunofluorescence performed in both laboratories.

Immunohistochemical staining of tissue section specimens was performed by incubation of fixed specimens in a 1:80 dilution of convalescent-phase horse serum containing antibody to *E. equi*. After washing in PBS, alkaline phosphatase–conjugated goat antibody to horse immunoglobulin was used as a secondary antibody. Tissue specimens were processed and evaluated by light microscopy as previously described [12].

### PCR Testing for *B. burgdorferi*, *B. microti*, and the HGE Agent

Postmortem whole blood samples were collected in EDTA. PCR testing of whole blood specimens for the HGE agent was performed as previously described [2, 13]. PCR assays for *B. burgdorferi* and *B. microti* were performed with use of a concentrated lysate of whole blood according to a modification of previously described conditions [11, 14]. The modified procedure included total cell lysis and concentration of a 500-mL aliquot of whole blood in a hypotonic lysis buffer containing nonionic detergent (Isoton II [Coulter, Hialeah, FL] and Triton X100 [Sigma, St. Louis]). The blood aliquot was added to a 2-mL nonsiliconized microcentrifuge tube containing 800 mL of Isoton II; after the addition of 150 mL of 10% TX-100, the mixture was vortexed for 3 seconds and then centrifuged at maximum speed (>12,000g) for 1 minute. The supernatant was removed and discarded, leaving a small cellular pellet. Nucleic acid extraction was carried out by means of a modified version of IsoQuick (Orca Research, Bothell, WA), and amplification and detection were performed as previously described [11, 14].

### Results

An autopsy was performed within 72 hours of the time of death. Both lungs were found to be congested (weight of right lung, 660 g; weight of left lung, 550 g); histological evaluation of a lung tissue specimen showed mild-to-moderate hemorrhagic pulmonary edema and pleuritis. The spleen was enlarged (weight, 580 g) and contained numerous polymorphonuclear leukocytes that were located primarily within septic foci. Examination of the heart revealed no significant coronary artery disease; however, severe widespread transmural myocarditis with endocardial involvement was observed, as were mixed neutrophilic and lymphocytic infiltrates. Scattered foci of myocardial necrosis were present. Cellular infiltrates were found in the sinus node, atria, ventricles, and areas adjacent to but not including the atrioventricular node. Lymphocytic infiltrates were seen in the bundle and adjacent to the bundle branches but did not involve the ventricular conduction system.

Serological and PCR evaluations for known deer tick–transmitted diseases were performed on postmortem specimens of serum and clotted whole blood, respectively, as previously described [2, 11, 13, 14]. At testing laboratories, serum titers of IgG and IgM antibodies to *B. burgdorferi* were negative by means of a conventional ELISA for IgG antibody and western blotting, immunoassays for IgG and IgM antibodies to recombinant *B. burgdorferi* antigen, as well as a sensitive indirect immunofluorescent antibody IgM assay for early Lyme disease. Direct immunofluorescence for detection of *B. microti* infection was also negative, as were PCR studies of serum and clotted whole blood specimens for both *B. burgdorferi* and *B. microti* [11, 14]. However, titers of antibody to *E. equi* were significantly elevated at >1:256 according to indirect immunofluorescence performed in both laboratories.

Consistent with the serological data, a 293-bp region of the 16S rRNA gene specific for the HGE agent was detected by PCR analysis of DNA extracted from a whole blood specimen collected after death, whereas multiple negative controls were nonreactive. A 487-bp noncontiguous PCR product specific to the HGE agent that was obtained from the blood sample was subjected to DNA sequencing and phylogenetic analysis to confirm the identity of the HGE agent; two sequence polymorphisms that distinguish the HGE agent from *E. equi* proper were detected at nucleotide positions 27 and 78 of the amplified sequence [13]. Immunohistochemical evaluation of a cardiac tissue specimen with horse antibody to *E. equi* showed rare inclusions within cells with mononuclear morphology that infiltrated the cardiac and epicardial tissues (figure 1). Other tissues examined with use of antibody to *E. equi* as well as cardiac tissue control specimens stained with immune serum were negative.

### Discussion

Although clinical cases of HGE have been described only recently, serological evidence of exposure to the HGE agent...
was present in serum specimens from patients with Lyme disease that have been stored since 1987 [15], and molecular evidence of infection with the HGE agent was found in deer ticks collected from Wisconsin in 1983 [2]. The delay in recognition of ehrlichiosis in the United States may be related in part to the fact that many cases appear to be asymptomatic or subclinical [16]. When symptoms are present, the typical presentation of an undifferentiated febrile illness (including fever, headache, malaise, myalgias, and arthralgias) may at times be difficult to distinguish from that of early Lyme disease, especially since both diseases have similar epidemiological distributions and are transmitted by the same tick [2, 17].

The diagnosis of acute HGE, during which serological testing is often not informative, is problematic. Laboratory findings of leukopenia, thrombocytopenia, and elevated transaminase levels are common in ehrlichiosis [1, 5]. The definitive diagnosis is based on in vitro cultivation of the agent; however, the sensitivity of culturing is uncertain, and results are obtained in several days to weeks. PCR-based tests are sensitive and capable of generating same-day results during acute infection, but these tests are not widely available. In the interim, until rapid and accurate tests are available, empirical administration of doxycycline should be encouraged for treatment of patients who are exposed in areas in which both organisms are endemic, since this drug is active against both \textit{Ehrlichia} species and \textit{B. burgdorferi}.

Although infection with the HGE agent in this case was well established, the pathogenesis of the associated carditis was less clear. Coinfection with \textit{B. burgdorferi}, which causes carditis in experimental mouse inoculation models, cannot be ruled out with absolute certainty since PCR testing is of relatively low sensitivity after therapy [14, 18]. Furthermore, concurrent ehrlichial infection may have ameliorated early humoral responses to \textit{B. burgdorferi} [19]. Simultaneous infection with \textit{B. burg-
Hofmeister and the HGE agent has been described in humans [20, 21]. Two cases of fatal carditis have been described in patients with Lyme disease [8, 22]. At least one of these cases was complicated by concurrent babesiosis [8]; it is not known whether ehrlichial coinfection was present in either or both of these cases.

The HGE agent was recently recovered from the blood of a patient with clinical pericarditis who had an inflammatory exudate in the pericardial fluid [23]; although we were able to demonstrate material of the HGE agent in the myocardium by immunostaining, we could not determine whether the effect of HGE is direct or indirect. An alternative pathogenetic mechanism could be activation of an underlying viral infection via transient but sometimes severe immune suppression and inflammatory cell dysfunction, which has been noted in animal models of infection with Ehrlichia [24, 25] and in some human cases [1]. A striking example of the immunosuppressive potential of acute ehrlichial infection is seen in the model of experimental infection in sheep with Ehrlichia phagocytophila and a tick-borne encephalitis virus (lopping ill virus); in this model, infection with both pathogens, each of which alone has low virulence, is required to produce viral encephalitis that is clinically apparent [26]. The frequency with which HGE itself is associated with carditis and the degree to which Lyme carditis or other manifestations of Lyme disease are influenced by concurrent HGE merit further evaluation.

Acknowledgments

The authors gratefully acknowledge the assistance of Val Hal ling, Lynne Sloan, and Jenifer Magera in the performance of serological testing.

References