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Cardiac Tropism of *Borrelia burgdorferi*

An Autopsy Study of Sudden Cardiac Death Associated with Lyme Carditis

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Fatal Lyme carditis caused by the spirochete *Borrelia burgdorferi* rarely is identified. Here, we describe the pathologic, immunohistochemical, and molecular findings of five case patients. These sudden cardiac deaths associated with Lyme carditis occurred from late summer to fall, ages ranged from young adult to late 40s, and four patients were men. Autopsy tissue samples were evaluated by light microscopy, Warthin-Starry stain, immunohistochemistry, and PCR for *B. burgdorferi*, and immunohistochemistry for complement components C4d and C9, CD3, CD79a, and decorin. Post-mortem blood was tested by serology. Interstitial lymphocytic pancarditis in a relatively characteristic road map distribution was present in all cases. Cardiomyocyte necrosis was minimal, T cells outnumbered B cells, plasma cells were prominent, and mild fibrosis was present. Spirochetes in the cardiac interstitium associated with collagen fibers and co-localized with decorin. Rare spirochetes were seen in the leptomeninges of two cases by immunohistochemistry. Spirochetes were not seen in other organs examined, and joint tissue was not available for evaluation. Although rare, sudden cardiac death caused by Lyme disease might be an under-recognized entity and is characterized by pancarditis and marked tropism of spirochetes for cardiac tissues. *(Am J Pathol 2016, 186: 1195—1205; http://dx.doi.org/10.1016/j.ajpath.2015.12.027)*

Lyme disease is caused by the spirochete *Borrelia burgdorferi* in North America and transmitted by certain species of *Ixodes* ticks. Approximately 30,000 cases are reported annually in the United States, but the actual number may be 10-fold higher.[1] Other *Borrelia* species in the *B. burgdorferi sensu lato* complex, including *Borrelia afzelii* and *Borrelia garinii*, cause Lyme disease in Europe. Clinical symptoms are largely dermatologic, neurologic, and musculoskeletal. In the United States, cardiovascular symptoms occur in approximately 1.1% of reported cases,[2] can manifest as conduction block,[3,4] and, when recognized, usually resolve with appropriate antibiotics.[5] Fatal myocarditis is rare, with

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only four cases reported before 2013 in the United States[6–8] and Europe,[9] including three cases with detailed pathology findings.[6–8] In 2013, the CDC reported three additional cases of fatal carditis associated with Lyme disease.[10]

The most frequently described pathologic findings in Lyme disease correlate with the clinical stages of disease.[11,12] In early localized disease (3 to 30 days after tick bite), skin biopsy specimens of erythema migrans show lymphohistiocytic perivascular dermatitis, whereas in late-stage disease (months to years after tick bite), synovial biopsy specimens for arthritis classically show synovium with villous proliferation, lymphoplasmacytic infiltrates, and microangiopathic change.[13] Lyme carditis occurs most frequently during early disseminated Lyme disease, generally days to weeks after tick bite. Endomyocardial biopsy specimens and autopsy findings show edema, subendocardial infiltrates (on biopsy specimen), pancarditis (on autopsy) with perivascular interstitial infiltrates, increased collagen deposition, and limited cardiomyocyte necrosis.[7,8,14,15] Largely European studies of central nervous system pathology during early disseminated to late-stage disease have described brain and spinal cord meningeal and perivascular infiltrates, gliosis, microglial activation and glial nodules,[16] involvement of cranial nerves[17] in addition to peripheral nerve infiltrates, and axonal degeneration.[18] Other fatal complications reported include a case of acute respiratory distress syndrome,[19] and neonatal death after transplacental transmission.[20,21]

By histopathology, spirochetes have been reported in skin,[22–24] synovium,[13] ligament,[25] spleen,[26,27] leptomeninges,[17] and heart[7,15,28] using silver stains. However, spirochetes often are not visualized, as documented in confirmed cases of neuroborreliosis,[16] fatal myocarditis,[6,8] and lymphocytoma cutis,[29] likely because of low numbers of spirochetes and high background seen in conventional silver stains. PCR can identify (or detect) *B. burgdorferi* DNA in tissue,[8,29] and, more recently, immunohistochemistry (IHC) has been used to show borrelial antigens in clinical specimens[30] and in experimental models.[31]

B. burgdorferi possesses numerous adhesins that bind host extracellular matrix molecules, including glycosaminoglycans, fibronectin, collagen, and the small proteoglycan decorin,[32] which mediate tissue tropism, pathogenicity, and immune invasion. In experimentally infected mice, *B. burgdorferi* binding to decorin has been implicated in spirochete cardiac tissue tropism.[31,33]

Here, we report the pathologic, immunohistochemical, molecular, and serologic findings of five cases of sudden cardiac death associated with Lyme disease and show marked tropism of spirochetes for heart tissue, providing insight into the pathogenesis of Lyme myocarditis. Partial data on three of the five patients have been published previously[10]; together, these and additional data are reported here and provide a broader description of a rare disease.

Materials and Methods

Patient Selection

Autopsy specimens were submitted to the CDC Infectious Diseases Pathology Branch and Bacterial Diseases Branch Laboratories in 2013 to 2014 by medical examiners and state public health laboratories. All cases with laboratory evidence of *B. burgdorferi* infection were included. A median of 3 (range, 1 to 5) cardiac specimens were submitted per case. Other tissues were evaluated as available and are described later.

Autopsy tissues from patients were submitted to the CDC as part of a public health investigation, and did not require review by an institutional review board. In an effort to maintain family confidentiality, data are presented in summary form and specific ages are not provided.

Histochemistry

Hematoxylin and eosin, Warthin-Starry (WS), and trichrome stains were performed according to standard protocols using Leica ST5020 multistainer (Leica, Buffalo Grove, IL), Dako Artisan Link autostainers (Dako, Carpinteria, CA), and manual methods, respectively. Slides for the WS stain were cut at 5-μm thickness to maximize detection of spirochetes.

Immunohistochemistry

IHC was performed using a polymer-based indirect immunoalkaline phosphatase detection system with a fast red chromogen for the colorimetric detection of an antibody/polymer complex (Biocare Medical, Concord, CA). *B. burgdorferi* IHC was performed using rabbit polyclonal antibody (immunized against a whole-cell preparation) (ab34970; Abcam, Cambridge, MA), used at a 1:1000 dilution with proteinase K pretreatment.[31] Internal validation data showed that this assay cross-reacts with *Borrelia hermsii* and *Treponema pallidum*, but does not detect *Leptospira* species (data not shown). A mouse monoclonal antibody against *B. burgdorferi* flagellin (clone H9724), used at a 1:2000 dilution (gift from Barbara Johnson, Division of Vector Borne Diseases, CDC) was used for confirmation (data not shown). To evaluate for cardiomyocyte injury, IHC for complement components C4d and C9 was performed as previously described.[34,35] Mouse monoclonal antibodies against CD3 (M7254; Dako) and CD79a (sc20064; Santa Cruz Biotechnology, Dallas, TX) were used at 1:100 and 1:500 dilutions, respectively. Rabbit polyclonal antidecorin (immunized against a recombinant protein epitope tag) (HPA003315; Sigma Aldrich, St. Louis, MO) was used at a 1:100 dilution.[36] To evaluate for co-infections, IHC for Heartland virus was performed as previously described.[37] IHC for Powassan virus used hyperimmune mouse ascitic fluid (courtesy of Pierre Rollin, CDC), which is known to detect Powassan virus antigens in formalin-fixed, paraffin-embedded (FFPE) human tissues at a 1:1000 dilution. IHC for *Anaplasma*

phagocytophilum used horse polyclonal antibody (gift from Richard E. Cortsvet, Louisiana State University), which is known to detect *A. phagocytophilum* in FFPE human tissues at a 1:1000 dilution.

PCR

DNA were extracted from FFPE tissues as previously described using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA).[38] DNA was extracted from whole blood using the QIAamp DNA Mini Kit (Qiagen) following the manufacturer's instructions. Primers and probes targeting outer surface protein A,[39] flagellin,[40] and plasminogen binding protein[41] were modified slightly and used for real-time PCR. PCR for outer surface protein A was performed in 25-μL volumes with 5 μL DNA extract using the QuantiTect Multiplex PCR Kit (Qiagen), and run on the Mx3005P QPCR System (Agilent, Santa Clara, CA) with cycling conditions of 95°C for 15 minutes, 45 cycles of 94°C for 1 minute, and 60°C for 1 minute. Positive controls included FFPE blocks containing cell culture isolates of *B. burgdorferi* (courtesy of Barbara Johnson, Division of Vector Borne Diseases, CDC). Samples were considered positive when the threshold cycle value was ≤40. PCR for flagellin and plasminogen binding protein was performed in 20-μL volumes with 5 to 10 μL of DNA extract using TaqMan Fast Universal PCR Master Mix (Life Technology, Grand Island, NY) and run on the AB 7500 FAST Dx Real-Time PCR instrument (Applied Biosystems, Grand Island, NY) with cycling conditions of 95°C for 20 seconds, 45 cycles of 95°C for 3 seconds, and 60°C for 30 seconds. Samples were considered positive if their threshold cycle value was ≤40 and less than the threshold cycle value of the positive control. PCR for *Babesia microti* was performed as previously described[42] to evaluate for co-infection.[7]

Serology

At the CDC, all serologic tests were performed using patient sera and following the manufacturer's instructions. Two enzyme immunoassays were used: a whole-cell sonicate enzyme immunoassay [Vitek Immunodiagnostic Assay System (VIDAS) Lyme IgM and IgG Polyvalent Assay, bioMérieux, Inc., Durham, NC] and the C6 *B. burgdorferi* (Lyme) enzyme immunoassay (Immunetics, Boston, MA). IgM and IgG Western Blots (WBs) (MarDx Diagnostics, Inc., Carlsbad, CA) also were run on all patient sera. The immunoblotting results were interpreted according to the guidelines proposed by the CDC,[43] in which 2 of the 3 bands and 5 of the 10 bands are required for positive IgM and IgG WBs, respectively.

Results

Demographic and Clinical Data

Summary data are presented in Table 1. Briefly, ages ranged from young adult to late '40s. Deaths occurred in states containing counties with a high or moderate incidence of Lyme disease (New York; New Hampshire, with recent travel to Connecticut; Massachusetts; and Indiana). Deaths occurred in the months of July (three deaths), August (one death), and November (one death) 2012 to 2014. Three patients were otherwise healthy. Two patients had known underlying disease: one patient had a history of Wolf-Parkinson-White syndrome, and another patient had a history of diabetes mellitus, hepatitis B, and cocaine and alcohol use. One patient had given birth approximately 6 months previously. All patients were reported to engage in outdoor activities, two patients had known exposure to ticks, and one patient reported a recent bite.

Table 1 Demographic and Clinical Data

Age range, years (median)	Young adult to late 40s (28)
Male sex/*n*	4/5
State (*n*)	NY (2), MA (1), NH (1; exposure in CT), and IN (1)
Month of death (*n*)	July (4), August (1), November (1)
Otherwise healthy/*n*	3/5
Known comorbidities	WPW (1); DM, HBV, and alcohol and cocaine use (1)
Tick exposure activity/*n*	4/5
Prodrome (*n*)	<3 weeks (3), several months (1)
Recent joint pain/*n*	3/5
Dermatologic lesion/*n*	0/5; possible spider bite (1)

DM, diabetes mellitus; HBV, hepatitis B virus; WPW, Wolf-Parkinson-White syndrome.

A prodrome was reported for each of the patients that included the following: nonspecific viral-like illness, malaise, shortness of breath, and anxiety. One of these patients also had joint and muscle pain, and the other two patients had joint pain for an unknown duration. No dermatologic lesion was documented or reported for any of the patients, although one patient was evaluated in an emergency department 1 month before death for an arm lesion diagnosed as a possible spider bite from which methicillin-resistant *Staphylococcus aureus* was isolated in culture.

One patient underwent pre-mortem serologic screening for Lyme disease and results were negative. Lyme disease was not clinically suspected in a second patient who sought care for episodic shortness of breath; the remaining two patients did not seek medical care. For two cases, Lyme disease initially was suspected by cardiac pathology at a tissue bank transplant service (no cardiac tissue was transplanted). The remaining two patients were diagnosed at the CDC through unexplained-death investigations.

Gross Pathologic Findings

At autopsy, hearts generally were enlarged (Table 2), one heart was described as diffusely mottled and soft and another heart had an odd muddled coloration with epicardial petechiae, and two patients had significant atherosclerosis. The patient with the smallest heart (380 g; normal, <350 g), who was otherwise healthy, also had the smallest body mass

Table 2 Pathologic Findings and *Borrelia* Testing

Parameters assessed	Findings	Normal values
Gross autopsy findings		
Heart weight, range (median)	380–716 (650) g	250–350 g
Heart weight, % range (median)	0.45–0.97 (0.62)	0.5%
Gross cardiac findings	Biventricular dilation (1) Biventricular dilation and moderate CAD (1) Concentric LVH and severe CAD (1) Biventricular hypertrophy (1) Concentric LVH, biventricular dilatation, and mild CAD (1)	
LV thickness, range (median)	1.4–2.1 (1.7) cm	0.6–1.1 cm
RV thickness, range (median); *n*	0.4–1.5 (0.5) cm; 4	<0.5 cm
Liver weight, range (median)	1.14–2.55 (2.1) kg	1.4–1.7 kg
Spleen weight, range (median)	170–440 (316) g	150–200 g
Microscopic findings		
Pancarditis with road map distribution, *n/N*	5/5	
Documented conduction system involvement, *n/N*	2/2	
Overt cardiomyocyte injury, *n/N*	1/5 (focal)	
Increased interstitial fibrosis by trichrome, *n/N*	5/5	
Cardiac C9 and C4d deposition by IHC, *n/N*	0/5	
T cells > B cells by IHC, *n/N*	4/5	
Histopathology of other organs (*n/N*)	Leptomeningitis: mild (1/5), minimal (3/5) Mild portal lymphocytic infiltrates (2/4) Cirrhosis (1/4) Prominent splenic immunoblasts (1)	
Results of *Borrelia* testing across tissues		
Heart-positive WS stain, *n/N*	5/5	
Heart-positive *Borrelia* IHC, *n/N*	5/5	
Heart-positive *B. burgdorferi* PCR, *n/N*	5/5 (*ospA* and *flaA*); 3/5 (*pbp**)	
Other organs positive for *Borrelia* IHC, *n/N*	CNS (2/5), liver (0/4), lung (0/5), kidneys (0/4), spleen (0/4), skin (0/1), prostate (0/2), synovium was not available	
Other organs and whole blood- positive *B. burgdorferi* PCR, *n/N*†	Liver (1/4), whole blood (1/1), CNS (0/5), lung (0/5), kidneys (0/4), spleen (0/4), skin (0/1), synovium was not available	

For findings in which $N < 5$, not all patient samples were available for testing.

*Sample was positive by *pdp* PCR, but the positivity was lower than the positive control.

†Other organs tested by *ospA* PCR; whole blood was tested by *flaA* and *pdp* PCR.

CAD, coronary artery disease; CNS, central nervous system; *fla*, flagellin protein gene; IHC, immunohistochemistry; LV, left ventricle; LVH, left ventricular hypertrophy; *ospA*, outer surface protein A gene; *pbp*, plasminogen binding protein gene; RV, right ventricle; WS, Warthin-Starry.

(61 kg), with relative cardiomegaly with a cardiac mass percentage of 0.62% (normal, 0.5%[44]). The active outdoor enthusiast had a large heart (570 g), but a 125-kg body mass, for a normal cardiac mass percentage of 0.46%. The patient with a clinical history of Wolf-Parkinson-White syndrome had a 734 g heart (cardiac mass percentage, 0.74%) with 95% stenosis of the right coronary artery, 90% stenosis of the left anterior descending coronary artery, and concentric left ventricular hypertrophy. One patient had 60% stenosis of the left anterior descending coronary artery with biventricular dilation, and another patient had focal mild atherosclerosis with biventricular dilation. Mild hepatosplenomegaly was seen in all cases.

Microscopic Findings

The cardiac histopathology of these patients was remarkably uniform. At low magnification, a characteristic pattern of intersecting curvilinear bands of interstitial inflammatory infiltrates was observed (Figure 1, A–F), extending from the endocardium to the epicardium. The infiltrate had a prominent perivascular distribution (Figure 1C). No conspicuous differences were appreciated between the infiltrates in the left ventricle, right ventricle, and septum. The conduction system specifically was evaluated in two cases: the atrioventricular and sinoatrial nodes were involved by inflammatory infiltrates in one case, and in another case, sections of the atrioventricular node showed intense necrotizing inflammation (Figure 1D). Coronary arteries were uninvolved by inflammation, although inflammatory infiltrates abutted arterial adventitia in the epicardial adipose tissue. The infiltrates comprised lymphocytes, histiocytes, and prominent plasma cells. Neutrophils and eosinophils were not present in significant numbers, and no multinucleated giant cells, granulomas, or vasculitic changes were seen. Scattered foci of cardiomyocyte contraction band necrosis

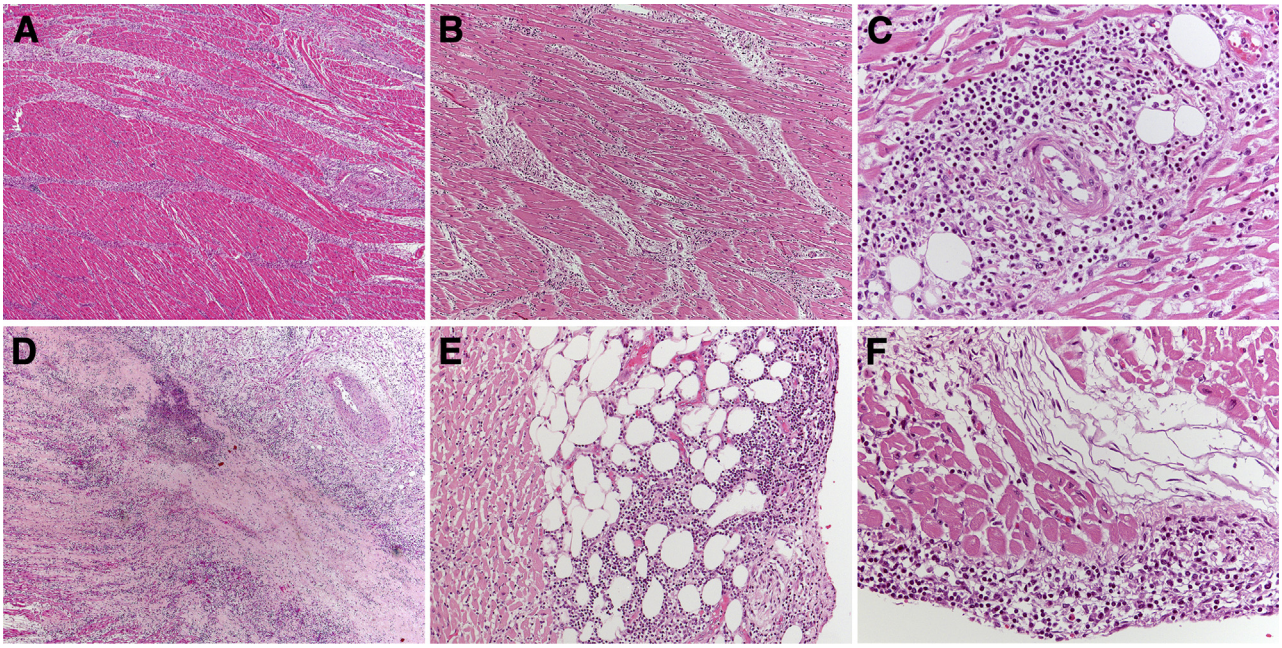

Figure 1 Pathology of Lyme myocarditis. **A**–**C:** Carditis with interstitial perivascular lymphoplasmacytic infiltrates in a road map distribution. **D:** Atrioventricular node. The atrioventricular artery is seen in the upper right. Infiltrates involve epicardial adipose tissue (**E**) and endocardium (**F**). Original magnification: ×6.25 (**A** and **D**); ×12.5 (**B**); ×25 (**E**); ×50 (**C** and **F**).

were evident in only a single case. Background interstitial fibrosis and cardiomyocyte hypertrophy was noted in one case (the patient with cirrhosis and diabetes).

One case had mild leptomeningeal lymphocytic infiltrates that focally involved cranial nerve roots, in addition to rare parenchymal perivascular infiltrates. Two additional cases had minimal focal leptomeningeal infiltrates. Mild portal lymphocytic infiltrates were present in liver in two cases, and nodular cirrhosis was present in the case with known hepatitis B virus infection. One case had prominent splenic immunoblasts. Sections of lung, kidney, nonlesional skin, and other organs as available (Table 2) showed no significant changes. No joint or synovial tissues were available for analysis.

Histochemical Findings

By WS stain, spirochetes were seen in the epicardium, myocardium (within bands of interstitial inflammation and fibrosis) (Figure 2A), and endocardium; focally up to approximately 6 per high-power field. One case, after PCR diagnosis, required evaluation of multiple blocks and levels to detect a single convincing spirochete by WS stain. No spirochetes were seen in other tissues by WS stain. Trichrome stains showed increased cardiac interstitial collagen deposition in all cases, particularly in the single case with underlying interstitial fibrosis.

Immunohistochemistry

By IHC, spirochetes were noted within the myocardial interstitial infiltrates (Figure 2B), in the subendocardium, and occasionally in pericardial tissue in association with lymphohistiocytic infiltrates. No spirochetes were seen within coronary arteries or their branches. Spirochetes were associated with collagen fibers, often apposed along the length of the fibers. Although generally localized in the inflamed interstitium, spirochete density did not appear to have any correlation with the density of inflammatory infiltrates (either within a case or between cases). Compared with WS stain, approximately 5- to 10-fold more spirochetes were visualized by IHC in sections of cardiac tissue.

Brain was examined in all patients, but only rare spirochetes were seen by IHC in areas of mild inflammation in two of the five cases (Figure 3, A and B). No spirochetes were seen in sections of lung, liver, kidney, spleen, gastrointestinal tract, prostate, or nonlesional skin. Although no joint tissue was available, other soft tissues were examined closely, including fibroadipose and fibroconnective tissue, visceral pleura, splenic and hepatic capsules, and respiratory cartilage.

IHC was negative for cardiomyocyte complement component C4d or C9 in all cases. CD3-positive T cells outnumbered CD79a-positive B cells in four of the five cases, in an approximate 3:2 ratio; the other case had T and B cells in equivalent numbers. Vague nodularity of T-cell and B-cell infiltrates occasionally was observed, but lymphoid follicles were absent in the cardiac inflammatory infiltrates. Immunohistochemical assays for Heartland virus (spleen, $n = 3$; lymph node, $n = 1$) and Powassan virus (central nervous system, $n = 5$) were negative.

Decorin protein localized to the cardiac interstitium in areas of collagen deposition (Figure 4, A and B). On serial sections performed on all cases, spirochetes co-localized

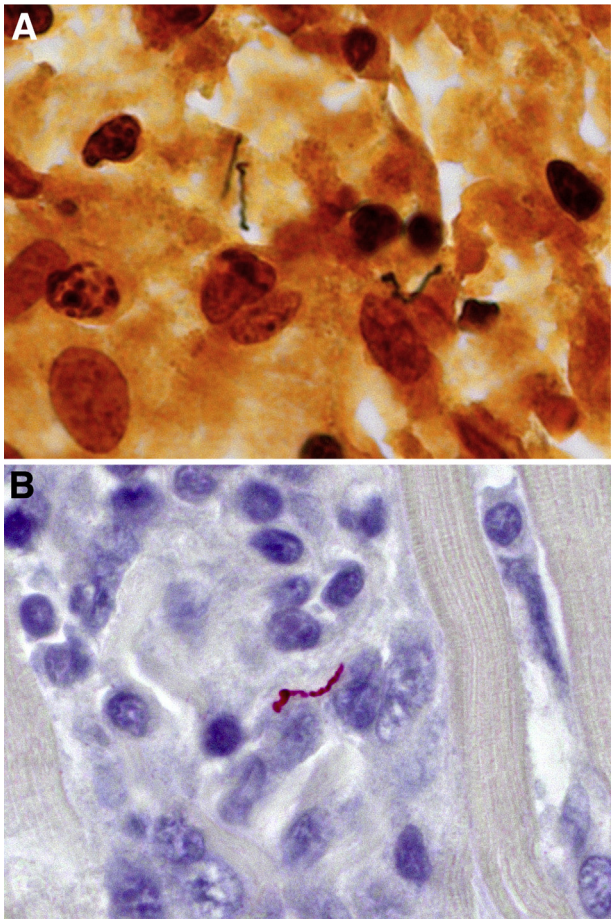

Figure 2 Spirochetes within cardiac tissue. Spirochetes are seen by Warthin-Starry stain (**A**) and *Borrelia* species immunohistochemistry (**B**). Original magnification, ×158 (**A** and **B**).

with collagen fibers and decorin protein (Figure 4, C–E). Decorin immunostaining also was noted in the leptomeninges. Of note, extensive decorin immunostaining also was seen in the collagen-rich prostate interstitium, in addition to splenic and hepatic capsular tissues, in the absence of spirochetes.

Molecular Studies

Heart tissue was positive for *B. burgdorferi* by real-time PCR in five of five cases, with one or more of three targets (Table 2). In addition, one available blood sample was positive. The central nervous system was negative by PCR in all cases examined, including from the blocks that had rare spirochetes visualized by IHC. PCR was positive in the liver of one patient. This was the same patient with PCR-positive whole blood who had pre-existing cirrhosis. Multiple sections of this liver specimen subsequently were examined by IHC and remained negative for spirochetes despite extensive evaluation of bands of fibrosis and the hepatic capsule. Molecular testing for *B. microti* was negative in all patients (heart, $n = 4$; spleen, $n = 1$).

Serology

Post mortem serum from all five cases tested positive for Lyme disease according to two-tier criteria (Table 3). One sample met both IgM and IgG WB criteria, with two of the three IgM bands and 6 of the 10 IgG bands reactive. The four remaining samples were positive by IgM WB criteria only, although three were nearly IgG positive with 4 of the 10 bands reactive. Of note, the patient with the positive IgG was the same patient who had the greatest ratio of B cells to T cells in the myocardial infiltrates.

Discussion

Sudden cardiac death associated with Lyme carditis is a rare presentation of a common disease; this study doubles the number of reported cases in the literature. A striking aspect of this case series is that all five patients had remarkably similar clinical and pathologic presentations. The findings support the proposed disease mechanism of spirochete cardiac tropism during early disease dissemination, the infiltration of cardiac tissue by inflammatory cells, and involvement of the conduction system, which likely mediates sudden cardiac death.

Considering these data, a major unanswered question is why sudden cardiac death during Lyme disease is so rare. The pathogenesis likely involves spirochete cardiac tissue tropism, and both host and spirochete factors may contribute to susceptibility. Although it is likely an underdiagnosed entity, underdiagnosis alone does not explain the disease rarity.

Lyme carditis occurs more frequently in men,[2,3] and four of five sudden cardiac deaths described here, and the previously reported four, occurred in men. Sudden cardiac

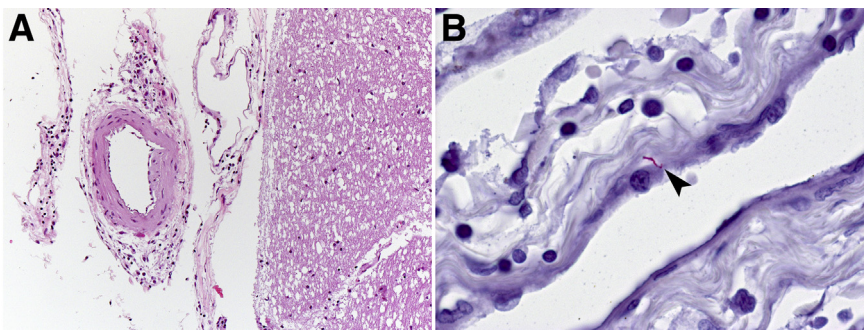

Figure 3 Neuropathology. Brain with mild leptomeningeal lymphocytic infiltrates (**A**) and rare spirochetes (**arrowhead**) by *Borrelia* species immunohistochemistry (**B**). Original magnification: ×25 (**A**); ×100 (**B**).

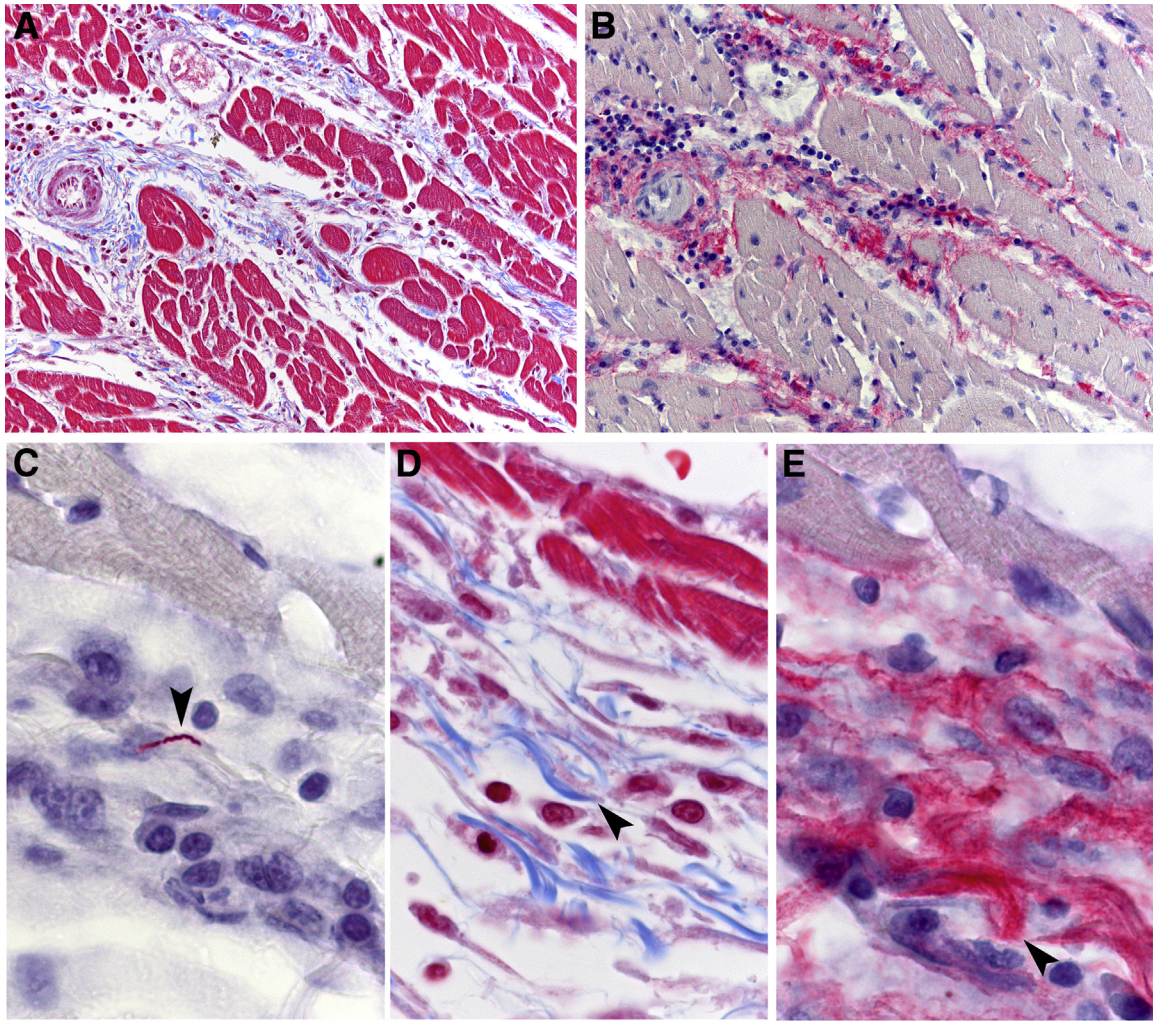

Figure 4 Localization of spirochetes with collagen and decorin. **A:** Lyme carditis cases show increased interstitial collagen by trichrome stain (blue). **B:** Cardiac decorin protein is localized to the interstitium by immunohistochemistry (red). **C:** Spirochetes by immunohistochemistry (IHC) (red, **arrowhead**) are seen along clear refractile collagen fibers. Collagen fibers are highlighted by decreasing the optical condenser aperture diaphragm (not shown). On consecutive sections, collagen fibers stain blue (**arrowhead**) by trichrome (**D**) and label with decorin (red, **arrowhead**) by IHC (**E**). Original magnification: ×50 (**A** and **B**); ×100 (**C**, **D**, and **E**).

death in general occurs more frequently in men,[45] and Wolf-Parkinson-White syndrome, a cardiac conduction system abnormality present in one of these patients, also is more common in men. A study in Slovenia found that although women present with predominantly cutaneous Lyme disease, men present with noncutaneous disease.[46] The influence of sex on infectious disease is complex, and may involve differences in hormones,[47] among other factors including behaviors associated with tick exposure. Of note, all patients also were <50 years of age; Lyme carditis has been reported to be more common among men aged 20 to 39 years and women aged 25 to 29 years.[2]

Table 3 *Post Mortem Borrelia* Serologic Data

Patient	VIDAS EIA result	VIDAS EIA value*	C6 EIA result	IgM WB interpretation	IgM reactive bands	IgG WB interpretation	IgG reactive bands
1	NA	NA	Positive	Positive	23, 39, 41	Negative	23, 41, 58, 66
2	Positive	4.74	Positive	Positive	23, 39, 41	Negative	41
3	Positive	5.57	Positive	Positive	23, 39, 41	Negative	23, 39, 41, 45
4	Positive	5.86	Positive	Positive	23, 39, 41	Negative	23, 41, 58, 66
5	Positive	5.46	Positive	Positive	23, 41	Positive	23, 39, 41, 45, 58, 66

Cut-off values were as follows: negative, <0.75; equivocal, ≥0.75 to <1.00; and positive, ≥1.00.
*VIDAS Lyme IgM and IgG polyvalent assay by bioMérieux, Inc.
EIA, enzyme immunoassay; NA, not available; VIDAS, Vitek Immunodiagnostic Assay System; WB, Western blot.

The presence of underlying heart disease might be an additional risk factor for Lyme carditis and/or sudden cardiac death. Significant underlying heart disease was present in two patients, and an additional patient had moderate atherosclerosis discovered at autopsy. In the other two patients, who were otherwise healthy, a degree of physiological cardiac stress likely was present: the woman had given birth 6 months previously and the man was a physically active outdoor enthusiast.

After myocardial injury, cardiac myocytes undergo cell necrosis and are replaced by scar composed of extracellular matrix.[48] There is a delicate balance between extracellular matrix synthesis and degradation for optimum remodeling of scar tissue to obtain near pre-insult strength. Regulation of the fibroblast and myofibroblast response is performed in part by several extracellular matrix proteins. One of these proteins, decorin, is a ubiquitous proteoglycan associated with type I and type II collagen-rich tissues.[49] Decorin binds collagen and affects collagen matrix structure and function.[50] Decorin affects fibroblasts by inhibiting transforming growth factor-β signaling, and is down-regulated after initial insult to allow for scar formation.[51,52] Decorin expression is up-regulated as fibrosis wanes and the physiological remodeling process occurs. In mouse models, decorin is essential for normal fibrotic remodeling after myocardial infarction.[53] Decorin expression in heart tissue also increases after experimentally increased ventricular afterload in mice, and decreases after physiological remodeling is complete.[54] In humans, decorin levels are increased markedly in myocardium after placement of left ventricular assist devices prior to transplant.[55] Although the precise chronology of the decorin increase in human myocardium after insult needs to be elucidated, it is clear that decorin is present in heart tissue during normal physiological remodeling.

B. burgdorferi spirochetes adhere to the extracellular matrix during disseminated infection, and decorin plays a key role.[56] Decorin binding is mediated by *B. burgdorferi* decorin binding proteins, in particular decorin binding protein A, which is a 20-kDa surface protein.[57] The dependence on decorin binding for the spirochete to experimentally infect the heart is striking. Decorin binding protein A is necessary for cardiac localization in a murine model,[58] and, conversely, cardiac infection is diminished in decorin knock-out mice.[59] Spirochetes co-localize with decorin in murine myocardium,[31] as was observed in the autopsy tissues examined here. This case series also showed marked cardiac tropism relative to other organs, as observed in the mouse models. Of note, decorin is expressed in other organs, including lung, liver, kidney, and prostate[36,49]; these visceral organs generally are not involved in Lyme disease, and were negative by *Borrelia* IHC in this study. The reasons for this are unclear. As a proteoglycan, decorin can have varied glycosaminoglycan chains attached to the protein core.[60] It is possible that cardiac-specific modifications of glycosaminoglycan groups on the decorin protein may alter *B. burgdorferi* spirochete adhesion, as seen with another pathogen, *Plasmodium falciparum*, in which the placenta-specific sulfation pattern of the chondroitin sulfate A glycosaminoglycan contributes to placental sequestration.[61]

Both spirochete and host genetic factors also may contribute to disease risk. *B. burgdorferi* genetic variation has been associated with virulence in humans,[62] and spirochete cardiac localization in mice is influenced by genetic variation in *dbpA*.[33] In this study, cultures could not be obtained for typing; future studies may use molecular methods. Little is known of human genetic risk factors for complications of Lyme disease. A Toll-like receptor-1 polymorphism has been associated with antibiotic-refractory Lyme arthritis,[63] and Toll-like receptor-2−deficient mice had a markedly increased spirochete load in tissues.[64] On the other hand, genetic associations with cardiac conduction abnormalities are well documented,[65] and might contribute to risk of conduction abnormality and/or sudden death during Lyme carditis.

Carditis generally is considered a manifestation of early disseminated Lyme disease. Our finding that four of the five patients were seropositive by IgM but not IgG WB criteria is consistent with this view. The greater number of cardiac T cells than B cells seen in the majority of patients might be a feature of the host response during the early disseminated stage of Lyme disease. In early erythema migrans, the dermal infiltrate is predominantly T cells.[66,67] B-cell infiltrates with germinal centers and evidence of pseudoclonality also can be seen later in the disease course,[68] and can reach high densities, where, particularly in Europe, infections can mimic B-cell lymphoma.[69] B cells also are prominent in arthritis and neuroborreliosis seen in later stages of disease. In mouse models during early infection (1 to 4 weeks after inoculation), myocardial infiltrates comprise T cells and few B cells.[70] In a nonhuman primate model of Lyme carditis, cardiac plasma cells, tissue IgG, and IgM deposition, and increased levels of the B-cell chemoattractant chemokine CXCL13 were observed and persisted for months to years after inoculation.[71] In this case series, the single patient with positive Lyme IgG serology also had the greatest ratio of cardiac B cells to T cells.

Early diagnosis and prompt treatment for Lyme carditis can be life-saving. Health care professionals should evaluate all patients with suspected Lyme disease for cardiac signs and symptoms, and obtain an electrocardiogram promptly if carditis is suspected. Similarly, providers should consider Lyme disease in patients who have cardiac symptoms and exposure in an endemic area. Diagnosis is based on clinical suspicion and serologic testing, with the caveat that serology testing may be falsely negative in a patient with recent illness onset.[72] Cardiac biopsies are generally not indicated for the primary diagnosis of Lyme carditis. Information on Lyme disease prevention is available from the CDC (Lyme Disease, *http://www.cdc.gov/lyme*, last accessed November 18, 2015).

The differential diagnosis for acute myocarditis is broad and includes diverse viral, bacterial, and protozoal agents, including enteroviruses and human parvovirus B19, although often no etiologic pathogen can be identified by specialized studies.[38] Postinfectious, postinflammatory, autoimmune, or drug hypersensitivity phenomena also can cause mycoarditis. The histopathology of the inflammatory infiltrates in Lyme carditis is relatively characteristic. To increase awareness among pathologists and medical examiners, we would like to coin the term "road map" to describe the pattern of intersecting curvilinear bands of interstitial infiltrates seen on low-power magnification. Although this distribution of infiltrates is similar to that seen in hypersensitivity myocarditis,[73] no granulomas, vasculitis, or significant eosinophilic infiltrates were seen in these Lyme carditis cases. To diagnose Lyme disease in cardiac specimens, the WS stain may be insufficiently sensitive to detect spirochetes and risks false positives owing to high background staining (silver-stained nerve fibers often resemble spirochetes). IHC using a commercially available antibody is a sensitive screening assay, and PCR on FFPE tissues allows for molecular identification and confirmation. Future studies are needed to better understand the incidence of Lyme carditis–associated sudden cardiac death, in addition to investigating mechanisms of spirochete tissue tropism in humans.

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