Long-term Follow-up of Patients with Persistent Lyme Encephalopathy

Brian A. Fallon, MD, MPH (Conference Co-Director) / Chandra A.

Background. Prospective studies of patients with early Lyme disease followed over time suggest that approximately 10% develop long-term problems. The long-term status of those who present with post-treatment chronic symptoms after getting Lyme disease is not known. Retrospective studies indicate that a risk factor for poor long-term outcome is delayed onset of treatment. Although Four U.S. Randomized Clinical trials have been conducted of those with chronic symptoms (most of whom were first treated late in illness), none has yet followed patients over time to assess long-term outcome. This presentation first will review all studies that have followed patients prospectively over time, including those in the U.S. and in Europe. Secondly, this presentation will present results from a long-term follow-up of patients with post-
Methods. The patient sample was drawn from the Brain Imaging and Treatment Study of Post-treatment Lyme Encephalopathy at Columbia University which enrolled 18 healthy volunteers and 37 patients with well-documented prior Lyme disease previously treated with IV ceftriaxone and current objective cognitive impairment. As participants were enrolled between 2000 and 2004, the follow-up interval was 8-13 years later. Follow-up assessments included a structured telephone interview as well as self-report questionnaires. The structured telephone interview asked patients to rate themselves on global improvement, on specific improvement (pain, fatigue, and cognition), on current functional impairment, and the extent to which they feel currently impacted by the earlier Lyme infection. The self-report questionnaires matched questionnaires given to patients prior to initial study enrollment and after completing study treatment 10 years earlier. Enrollment in this follow-up study continues. This report will be a preliminary assessment of the initial cohort who have been reached – 24 Lyme patients and 11 controls. We anticipate additional subjects will be included in time for the Conference presentation.
Immune suppression during infection of mice with Borrelia burgdorferi
Nicole Baumgarth, DVM, PhD

The immune response to Borrelia burgdorferi (Bb), which causes Lyme disease, is an enigma. Acute infections induce many different antibodies, some of which are used in diagnostic tests. These antibody responses appear initially strong, but seem to rapidly diminish following antibiotic treatment. In addition, mice and humans do not seem to develop protective immunity following the infection, as reinfections can occur frequently in endemic areas and experimental settings. Based on these findings we hypothesize that infection with Bb may suppress or subvert the development of protective long-term antibodies.

Previously we showed that lymph nodes are an early target of Bb infection in mice. We can find Bb in these lymph nodes, which become enlarged and lose their normal structure. Normally in infections, structures called germinal centers form in lymph nodes to produce long-lived antibody-producing cells, but in these Borrelia-infected lymph nodes, germinal centers appear only briefly despite an ongoing infection. We have found that these germinal centers are structurally abnormal and produce neither long-lived antibody-producing cells nor memory B cells. By vaccinating Bb-infected mice with a usually strong antigen, we showed that the lack of long-term immune response to Bb is because the infection actively suppresses the immune response.

Together these studies show that Borrelia burgdorferi evades mammalian immune responses by affecting germinal centers. The data explain the lack of long-term protective immunity to Bb in both experimentally-infected mice and humans and suggest that acute Bb-infection causes a state of immune suppression.
The currently recommended method for diagnosing Lyme disease is a two-tiered serology-based test that is limited in its ability to differentiate active from previous infection, diagnose early LD, and is not standardized among laboratories. Moreover, the interpretation of test results is open to subjectivity. Thus, a fresh approach for improved diagnostic development is critical to the field. Studies applying the global evaluation of small molecule biomarkers, “metabolomics”, for the discovery of biosignatures and biomarkers for monitoring cancers and metabolic diseases have shown tremendous promise. Although this technology has not been widely applied for infectious diseases, it is recognized that infectious diseases are manifested based on alterations in the biochemistry of a biological system.

Over the past three years our laboratory in collaboration with the Centers for Disease Control and Prevention, and New York Medical College successfully developed a serum based metabolomics approach to identify a small molecule biosignatures that can differentiate early Lyme disease (localized and disseminated) patients from healthy individuals from both endemic and non-endemic regions in the United States, and from patients with diseases that have look-alike symptoms or that are cross-reactive with existing serology-based tests for Lyme disease. This biosignature was developed with well-characterized retrospective samples and has significantly enhanced sensitivity as compared to the two-tiered serology-based testing of the same samples. These analyses provide proof-of-concept that a Lyme disease multi-analyte small molecule diagnostic can be developed. Further validation of this model; structural identification of biosignature metabolites; and additional comparisons targeting early localized versus disseminated Lyme disease, baseline versus 1-month post-treatment, and early Lyme disease versus Lyme arthritis are being performed. These efforts not only hold potential for new Lyme diagnostic and prognostic tools, but will also provide new information about the biology of Lyme disease.
Lyme Disease, caused by infection with the tick borne spirochete Borrelia burgdorferi, is a growing societal concern, especially in endemic regions of the United States and Europe. Part of the concern is rooted in the uncertainty surrounding pathological outcomes associated with B. burgdorferi infection. A large percentage (70%) of infected individuals develop the characteristic bulls-eye rash erythema migrans at the site of the infected tick bite, with progression to further clinical complications following dissemination of the spirochete. Lyme arthritis is the most common symptom, occurring in 30-60% of infected individuals. Part of the wide variation in Lyme disease symptoms and severity observed within the patient population is thought to be due to differences in heritable genetic risk factors.

To identify regulatory genes, we have used an unbiased forward genetic approach, based on the observation that genetic differences between inbred mouse strains lead to consistent differences in their Lyme arthritis severity. Early studies identified the location of large regulatory regions of the genome responsible for this effect, followed by a process of refinement through genetic mapping to identify candidate regulatory genes to be formally investigated.

We have recently reported our identification of the first naturally occurring gene polymorphism that regulates Lyme arthritis severity, in the lysosomal enzyme beta-Glucuronidase (Gusb). Our severely affected mouse strain carries a partial deficiency in this gene, which does not cause spontaneous disease but leads to a more severe inflammatory arthritis following infection with B. burgdorferi, consistent with a two-hit model. The Lyme arthritis severity of this strain was profoundly reduced after their partial Gusb deficiency was corrected through transgenic overexpression. We also found that partial Gusb deficiency increased the severity of rheumatoid arthritis in our mice, indicating a conserved role and a common mechanism. The human Gusb gene is polymorphic, and GUSB enzyme levels vary by more than 30-fold in the normal human population. Severe Gusb deficiencies are very rare and cause a congenital lysosomal storage disease called Sly syndrome,
characterized by pathological accumulation of the natural substrates of the GUSB enzyme, glycosaminoglycans (GAGs). We observed a pronounced increase in the accumulation of GAGs in the inflamed joint tissues of B. burgdorferi infected mice with partial or severe Gusb deficiencies, which may represent a novel mechanism underlying the pathogenesis of disease in Lyme and rheumatoid arthritis.

Lyme Disease – Induced Polyradiculopathy Mimicking Amyotrophic Lateral Sclerosis
Ahmet Z. Burakgazi, MD

Lyme disease, the leading arthropod-borne infection in the USA, can cause a wide spectrum of neurological conditions affecting central and peripheral nervous systems (PNS). Lyme disease related PNS manifestations include cranial neuropathy, polyradiculopathy, motor neuropathy, brachial plexopathy, lumbosacral plexopathy, and distal axonopathy. Lyme disease can cause severe, predominantly axonal polyradiculopathy, but it is less common than a chronic predominantly sensory polyradiculopathy. Furthermore, its diagnosis can be very challenging and it can mimic other neurological disorders such as Amyotrophic Lateral Sclerosis (ALS), or Guillain-Barre syndrome (GBS). Careful and detailed examination and investigation are required to confirm the diagnosis and to prevent misleading inaccurate diagnoses.

Herein, we report a unique case with an ALS-mimicking predominantly motor polyradiculopathy presentation caused by Lyme disease. A 64 year-old man presented with a one-month history of rapidly progressive weakness involving bulbar, upper limb and lower limb muscles. The physical examination showed widespread weakness, atrophy, fasciculation and brisk reflexes. The initial electrodiagnostic test showed widespread active and chronic denervation findings. The initial physical and electrodiagnostic findings were suggestive of ALS. However blood serology indicated possible Lyme disease. Thus, the patient was treated with doxycycline. The clinical and electrodiagnostic findings were resolved with the treatment.
The diagnosis of Lyme disease can be very challenging and it can mimic other neurological disorders such as ALS or GBS. Careful and detailed examination and investigation are required to confirm the diagnosis and to prevent misleading inaccurate diagnoses.

Promising Progress in Developing Protective and Therapeutic Approaches Against Anaplasma phagocytophilum Infection

Jason A. Carlyon, PhD

Human granulocytic anaplasmosis (HGA) is an emerging tick-borne disease in the United States, Europe, and Asia. The number of HGA cases reported to the U. S. Centers for Disease Control and Prevention rose nearly seven-fold between 2003 and 2012. Yet, seroprevalence data indicate that the infection is underreported in some endemic regions. HGA is an acute illness characterized by fever, chills, headache, malaise, leukopenia, thrombocytopenia, and elevated liver enzymes. Complications include shock, seizures, pneumonitis, rhabdomyolysis, hemorrhage, increased susceptibility to secondary infections, and death. Risk for complications and fatality is greater for the elderly, the immunocompromised, and when proper diagnosis and/or antibiotic therapy are delayed. The same ticks that transmit HGA also transmit Lyme disease, and coinfections do occur.

The causative agent of HGA is Anaplasma phagocytophilum, a bacterium that infects white blood cells called neutrophils. A. phagocytophilum is an “obligate intracellular” pathogen, which means that it cannot survive freely in the environment. Entry into host cells is therefore essential for its survival – a phenomenon that we are exploiting to develop effective preventative/therapeutic treatments against the disease.

A. phagocytophilum uses surface proteins called invasins to mediate entry into host cells. We identified three A. phagocytophilum invasins (OmpA, Asp14, and AipA) that are critical for infection. All are on the bacterium’s surface, which makes them
accessible to blocking antibodies. We delineated the specific regions of OmpA, Asp14, and AipA that are necessary for cellular invasion. A combination of antibodies against the “invasion domains” of all three proteins blocked A. phagocytophilum infection of host cells in vitro. We are now poised to evaluate these targets’ protective efficacies in vivo. Moreover, we have an exciting collaboration with Dr. Rich Marconi’s laboratory to combine our HGA protective epitopes with protective epitopes of Lyme disease targets identified by his group to develop a chimeric vaccine that will protect against both HGA and Lyme disease. Given the potential severity of these diseases, the limited choices of antibiotics for treating them, and the lack of a vaccine against either, our work is highly relevant.

TickChip Detection Microarray, Tickborne Viruses, STARI, and Transcriptome Profiling of Lyme Disease

Charles Chiu, MD, PhD

Lyme disease, a tickborne febrile illness caused by the bacterium Borrelia burgdorferi, is the most common vectorborne disease in the U.S, and incidence is increasing worldwide. An estimated 3.4 million commercial antibody-based tests are conducted annually in the U.S. for Lyme, yet these tests fail to diagnose up to 40% of acute Lyme disease cases. In addition, existing tests are unable to detect in a multiplexed fashion clinically significant co-pathogens in the tick vector, such as Babesia and Anaplasma. We will discuss the use of emerging genomic technologies – microarrays and deep sequencing – to address the urgent clinical need for improved Lyme diagnostics. First, a novel microarray platform called the TickChip has been developed and is able to detect all tickborne pathogens, including viruses, bacteria, and parasites from blood in a single assay. In preliminary data using spiked samples, we have shown that the TickChip has a sensitivity of detection of 1-10 genome copies of Borrelia
burgdorferi or Babesia microti per milliliter of blood. Second, whole-exome transcriptome profiling of Lyme disease patients by deep sequencing has identified a specific and robust host response signature for acute Lyme that persists for at least one month duration. This transcriptome data is currently being leveraged to design a multiplexed host response-based assay for acute Lyme disease diagnosis. Finally, we will report the use of unbiased deep sequencing for the detection and discovery of novel viruses in the hunt for the etiologic agent of STARI (Southern Tick-Associated Rash Illness), a Lyme-like illness that is spread by the Amblyomma tick, a vector which also harbors the pathogenic Heartland virus. The rising incidence and morbidity of Lyme and associated tick-borne illnesses demand the development of new diagnostics with the sensitivity to broadly detect these tickborne disease agents, both known and novel.

Sudden Cardiac Arrest Associated with Lyme Carditis

Joseph D. Forrester, MD, MSc

Lyme carditis is inflammation of the heart due to infection by Borrelia burgdorferi. It occurs uncommonly among patients with Lyme disease and is rarely fatal. In November 2012, a tissue bank pathologist detected pathology suspicious for Lyme carditis in tissues from a young adult who had died suddenly and unexpectedly. Seven months later, in July 2013, pathologists at a tissue bank and at CDC detected similar findings in heart tissues from two additional, unrelated young adults who had died suddenly. Medical records of the index cases were reviewed and specimens were tested for evidence of B. burgdorferi infection.

The decedents, two males and one female aged 26-38 years, were residents of Connecticut, Massachusetts, and New York. All had serologic evidence of early disseminated LD, and spirochetes were detected in cardiac tissues by microscopy, immunohistochemistry, and polymerase chain reaction assays. Donated corneas from two
decedents had been transplanted to three recipients before the diagnosis of Lyme disease was established, but no evidence of disease transmission was found.

Prompt recognition and early, appropriate therapy for Lyme disease is essential. Health-care providers should ask patients with suspected Lyme disease about cardiac symptoms and obtain an EKG if indicated. Conversely, they should ask patients with unexplained heart block about possible exposure to infected ticks. These three deaths underscore the need for better methods of primary prevention of Lyme disease and other tickborne diseases.

Disclaimer: The findings and conclusions in this summary are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Post-Antibiotic Persistence and Resurgence of Attenuated Borrelia burgdorferi

Emir Hodzic, DVM, PhD

Persistent infection with Borrelia burgdorferi, the agent of Lyme disease, is the rule, not the norm, in its many hosts. The ability to persist and evade host immune clearance poses a challenge to effective antibiotic treatment. There is growing experimental evidence in a variety of animal species that viable, non-cultivable spirochetes persist following treatment with several different antibiotics.

Our studies have undeniably found persisting spirochetes in mouse tissues 2, 4, 8 and 12 months after antibiotic treatment. The status of infection was evaluated based upon culture, qPCR, xenodiagnosis, and transplantation of allografts into naïve recipients. During the course of B. burgdorferi infection, an increasingly heterogeneous subpopulation of replicatively attenuated spirochetes arises that have lost small plasmids. These attenuated spirochetes remain viable, but divide slowly and are non-cultivable, thereby being tolerant to antibiotics. Treatment success is inversely correlated with spirochete populations, since spirochete burdens in mouse tissues are highest during early infection, when antibiotics work best. The
persistence of non-cultivable spirochetes occurs following treatment with several different classes of antibiotics. The most recent studies indicated that low numbers of persisting, but non-cultivable spirochetes resurge 12 months after antibiotic treatment, with re-dissemination into multiple tissues.

The phenomenon of persistence of non-cultivable spirochetes could be explained by antimicrobial tolerance, in which different classes of antibiotics fail to completely eliminate non-dividing or slowly dividing subpopulations of spirochetes. Plasmid loss is likely to occur during the course of infection and increase over time. The biological significance of attenuated spirochetes is probably insignificant, but the medical significance is another matter, and compels further investigation.

Xenodiagnosis of Lyme Disease in Humans

Linden Hu, MD

ABSTRACT

Background: Animal studies suggest that Borrelia burgdorferi, the agent of Lyme disease, may persist after antibiotic therapy and can be detected by various means including xenodiagnosis using the natural tick vector (Ixodes scapularis). No convincing evidence exists for the persistence of viable spirochetes after recommended courses of antibiotic therapy in humans. We determined the safety of using I. scapularis larva for the xenodiagnosis of B. burgdorferi infection in humans.

Methods: Laboratory-reared larval I. scapularis ticks were placed on 36 subjects and allowed to feed to repletion. Ticks were tested for B. burgdorferi by PCR, culture and/or isothermal amplification followed by PCR and electrospray
ionization mass spectroscopy. In addition, attempts were made to infect immunodeficient mice by tick bite or inoculation of tick contents. Xenodiagnosis was repeated in seven individuals.

Results: Xenodiagnosis was well tolerated with no severe adverse events. The most common adverse event was mild itching at the tick attachment site. Xenodiagnosis was negative in 17 patients with post-treatment Lyme disease syndrome (PTLDS) and/or high C6 antibody levels and in 5 patients after completing antibiotic therapy for erythema migrans. Xenodiagnosis was positive for B. burgdorferi DNA in a patient with erythema migrans early during therapy and in a patient with PTLDS. There is insufficient evidence, however, to conclude that viable spirochetes were present in either patient.

Conclusions: Xenodiagnosis using Ixodes scapularis larva was safe and well tolerated. Further studies are needed to determine the sensitivity of xenodiagnosis in patients with Lyme disease and the significance of a positive result.

“Understanding suicidal behavior risk in Lyme Disease: Perspectives from studies of suicidal behavior in depression.”

John G. Keilp, Ph.D.

Rates of both depression and suicidal behavior are elevated in chronic illnesses. It is well-known, in turn, that rates of depression are elevated in post-treatment Lyme disease syndrome (PTLDS). However, there is very little systematic research on suicidal behavior in PTLDS.

In our own work, we have found that PTLDS patients exhibit levels of depression that are intermediate between healthy individuals and those with true major depressions. Despite
their generally mild symptoms, a subgroup of PTLDS patients report suicidal thoughts. Outside of occasional case reports, though, little is known about the association of this thinking with actual suicidal behavior.

In addition, cognitive impairments in the context of depression are associated with increased risk for suicidal behavior, and a number of the cognitive impairments typically found in PTLDS are those associated with risk. These include impairments in memory and language fluency. While some components of attention are associated with risk for suicidal behavior in depression, and these components of attention are typically intact in PTLDS, those PTLDS patients who exhibit impairment in attention may be at elevated risk.

This talk will describe findings from our earlier study of PTLDS with memory impairment, findings from studies of risk factors for suicidal behavior in major depression, and propose recommendations for the evaluation of suicidal behavior in PTLDS. Standard clinical procedures for the assessment of suicidal behavior risk, as well as factors that may be unique to PTLDS, will be emphasized.

More than Lyme: Progress towards a tick-borne disease protective vaccine

Thomas N. Mather, PhD & Wendy Coy Shattuck

Worldwide, ticks can transmit more than 20 pathogens that impact human and animal health. In North America, just one tick vector, Ixodes scapularis, transmits at least 5 pathogens capable of causing significant disease. Costs to develop an effective and safe human vaccine targeting any one of these tick-transmitted pathogens are estimated to be more than $100 million. Discovery of a broad-spectrum vaccine that targets ticks but effectively suppresses pathogen transmission and host
infection by multiple tick-borne pathogens would represent a major milestone for improving public health. A strategy for inducing acquired resistance to the tick itself instead of to each individual pathogen they transmit represents a conceptually-sound and novel strategy for interrupting pathogen transmission and preventing a myriad of tick-borne diseases; empirical data in animal models and epidemiological data in humans strongly suggests that such a strategy can work. This presentation will discuss progress being made to develop a broadly-protective anti-tick vaccine.

While most vaccines are designed to promote antibody responses, a cellular immune response, particularly CD4+ (T helper) T-cell activity is required for vaccine efficacy. T-cells themselves are stimulated by a very limited number of highly specific antigenic determinants (epitopes) derived from the intruding organism’s proteins. Algorithms that accurately model the MHC-peptide interface have been central to the prediction of T-cell epitopes. With the availability of complete sequences of tick-secreted proteins (tick salivome), our studies to date have followed a path marked by milestones that include detecting and characterizing the nature of anti-tick immunity, identifying immune correlates of protection, critical antigen discovery and validation, and development of a novel animal model for accelerating translation into clinical studies. A total of 52 potentially antigenic candidate vaccine peptides have been identified and synthesized from over 150 tick salivary proteins. These are being used to construct prototype vaccines for proof of concept studies.

Case Presentation of a Suicidal Patient: Lyme or Depression?

Marina Makous, MD

A case will be presented that highlights key questions in the evaluation of the patient with treatment-resistant depression, suicidal thoughts, and possible Lyme disease. Identifying material has been changed to preserve patient confidentiality, but the doctor’s deliberations, clinical history, laboratory
results and outcome remain intact.

Atypical EM & PCR in Lyme Disease

Steven E. Schutzer, MD

Atypical Erythema Migrans has been observed in many suspected cases of Lyme disease in the past. Lyme disease was confirmed by CDC criteria of seroconversion in those cases. We applied a more direct confirmation by the use of a PCR-based assay. In the past estimates of atypical EM were on the order of 30%. Our recent data shows in a select population the cases approached 70%. Regardless of whether the number is 30% or 70% it is wise to consider that atypical, non bull’s-eye lesions, are not uncommon and may be first sign that an infection with Borrelia burgdorferi has occurred.

“The Impact of Lyme Disease on the Brain: Implications for Diagnosis, Treatment and Recovery”

Sheila M. Statlender, Ph.D.

This presentation will address the impact of tick borne diseases on the brain, and the critical role of mental health professionals in the diagnosis and treatment of the neuropsychiatric and neurocognitive symptoms that reflect brain involvement.

Hundreds of peer-reviewed studies have noted the ability of the Lyme disease bacterium to invade the central nervous system (neuroborreliosis), yet associated symptoms are often not acknowledged or adequately addressed. This may be compounded by an unfortunate tendency to attribute poorly understood or controversial medical illnesses to “stress,” “malingering,” or primary psychiatric disorders, ignoring the underlying medical etiology. Mental health practitioners with expertise in tick borne diseases understand the full range of associated neuropsychiatric and
neurocognitive symptoms, and play a vital role in: 1) screening for symptoms and relevant history as part of the psychotherapeutic assessment and treatment planning process, 2) making referrals for medical evaluation when indicated; 3) evaluating and identifying the presence of cognitive, sensory and other neuropsychological sequelae, “invisible” symptoms which could otherwise be overlooked; 4) providing supportive counseling and coping techniques; 5) facilitating appropriate communication between patients and their medical practitioners; and 6) participating in the development of safety plans for patients whose symptoms place them at risk for self-harm behaviors. The impact of Lyme disease on the brain must be identified and addressed in the overall treatment plan, in order to achieve optimal outcomes for these patients.

Powassan & Tick-Borne Encephalitis

Travis Taylor, PhD

Vector-borne flaviviruses, including West Nile virus, dengue virus and tick-borne encephalitis virus serocomplex (TBEV) greatly impact global health and cause millions of infections per year. These viruses are responsible for millions of infections annually, with symptoms ranging from severe encephalitis to hemorrhagic fever with mortality rates exceeding 30%. Currently, treatment is limited to supportive care and few vaccines are available. Powassan virus (a TBEV member) is endemic to North America and can share both vector (Ixodes scapularis) and reservoir host (Peromyscus) with Borrelia burgdorferi.

Despite severe disease in humans, infection of the natural vector and host has little noticeable effect. Based upon our previous work that revealed virus-specific antiviral genes in a TBEV-murine model, we predict that the coevolution of flaviviruses with their respective hosts has resulted in potent antiviral factors that suppress virus replication and protect the host from lethal infection, thus ensuring virus persistence.
This seminar will provide clinical information on diagnosis and treatment for Powassan virus and provide a background comparison to other flaviviruses. Current work to understand the host antiviral response to these viruses, as well as future therapeutic options will be discussed.

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**Borrelia miyamotoi**

*Sam R. Telford III, ScD*

Borrelia miyamotoi disease (BMD) has recently been described from febrile patients in the northeastern U.S. The agent has been known as a commensal of *Ixodes persulcatus* species complex ticks globally and may infect 1-5% of host seeking ticks in Lyme disease endemic sites. *B. miyamotoi* is likely maintained by transovarial transmission in ticks, although deer are reservoir competent as are white-footed mice. Chronic infection in immunodeficient mice causes spectacular hepatosplenomegaly with microabscesses and accumulations of spirochetes. Human cases have presented with meningoencephalitis or a flu-like illness confused with human granulocytic ehrlichiosis; elevated liver function tests and leukopenia were also seen in these patients. *B. miyamotoi* from North America may genetically differ from those in Eurasia, and unlike the prototypic Japanese strain, cannot be cultivated in vitro. The clinical spectrum and public health burden remain to be described. BMD is the 5th zoonotic infection transmitted by deer ticks in the northeastern U.S.

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**Pain Management**

*Michael L. Weinberger, MD*
In my talk I will discuss current and evolving issues in pain medicine and their relevance to Lyme disease including the use of ketamine and opioids for chronic pain.