CLINICAL REVIEW

Contemporary Issues on *Lyme Borreliosis* Management

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Introduction

The Center for Disease Control (CDC) estimates that 30,000 cases of Lyme disease are recorded yearly, establishing it as the most dominant tick-borne illness in the United States.[1] Despite these statistics, treatment algorithms for the various states of Lyme disease remain poor.[2] In addition, incidence of Lyme disease is inconsistently derived, especially in high-risk geographic regions. Some estimate the true incidence may be underestimated by a factor of seven.[3,4] Lyme disease infections are caused by the members of the *Borrelia* genus. This genus includes *B. afzelii, B. garinii,* and of course, the prototypical *B. burgforferi.* This bacterial genus invades humans via the bite of an infected Ixodes tick.[5,6]

Clinical Manifestations

Lyme disease classically manifests in three progressive stages: the acute phase, the early disseminated phase, and the late disseminated (LD) phase. In addition, there seems to be a poorly defined and controversial phase of the disease referred to as chronic Lyme disease (CLD). In the acute stage, patients typically experience flu-like symptoms, weakness, fever, chills, and rashes. The onset of acute Lyme disease is often heralded by the appearance of an erythema migrans (EM) rash. This EM rash adopts the shape of a "bull's eye", or a circular clearing localized entirely about the tick bite site with a necrotic center. Presence of EM rash confirms clinical acute Lyme disease.[6,7] This EM rash tends to subside regardless of antibiotic treatments.[8,9] Lack of adequate antibiotic treatment may result in the progression of early disseminated Lyme disease into the LD Lyme disease phase. The early disseminated phase and LD phase share several clinical features, including neurologic and cutaneous manifestations. In contrast, the appearance of arthralgias is generally more characteristic of LD disease, while cardiac and ocular manifestations typically occur in early disseminated disease.[10-13] Finally, a small subgroup of patients with documented Lyme disease may transition into a vaguely defined post- chronic lyme disease (CLD), characterized in by neurologic symptoms such as cognitive impairment and arthritic/systemic manifestations such as fatigue, joint pains, and exhaustion . The pathophysiology of CLD remains elusive. The very existence of the CLD is a matter of contention and many experts discourage routine serologic testing.[14] The presence of Lyme-directed antibodies in this chronic setting may not be the harbinger of a persistent microbial infection.[15,16]

Diagnosis

The diagnosis of Lyme disease is challenging. Physical exam is diagnostic during the acute phase due to the characteristic EM rash. Patients with both early disseminated and LD disease require serologic testing for disease confirmation.[17] This is because the clinical diagnosis of non-acute Lyme disease is nonspecific, as most of these findings are also observed in other conditions. The laboratory diagnosis of disseminated Lyme disease is arduous as coinfections with organisms such as anaplasmosis and/or babesiosis are common. Per CDC guidelines, a two-tier laboratory test remains the gold standard for the diagnosis of Lyme disease. This method uses an enzyme immunoassay, or 'EIA,' to measure the Lyme antibodies. Negative antibody test results should be an impetus to search for alternative diagnoses. The CDC recommends, a positive, or equivocal, initial antibody test should be followed by a confirmatory Western Blot assay for Lyme disease-specific IgM and IgG immunoglobins.[18] For patients whose symptoms exceed thirty days, the CDC recommends an IgG-only Western Blot analysis. As Lyme disease is the predominant tick-borne disease in North America, there is a strong need for a sensitive, specific, and reproducible diagnostic protocol. What follows, is an overview of some of these potential diagnostic issues.

<u>CD57 Antigen</u>

The CD57 antigen was once thought to be an accurate marker for human natural killer (NK) cells. However, the CD57 antigen is often absent in some NK cells. CD57 antigen may also be present on CD3+ T-cell subpopulation.[19] The CD3- CD57+ lymphocytes, to exclude T-cells expressing CD57 antigen appear to be terminally differentiated and poorly characterized lymphocytes. This cascade process culminates in CD57+ lymphocytes activation appears to signal a more potent cytotoxic capacity.[20] It is hypothesized that patients with acute Lyme disease have normal levels of CD57+ lymphocytes, whereas patients with CLD may have lower levels of these cells. The clinical significance and correlation between CD57+ cells and CLD, is tenuous. Some report levels of CD57+ lymphocytes do not differ significantly between CLD, healthy controls and patients who have recovered from Lyme disease.[20] Others report decreased levels of CD57+ lymphocytes in CLD in patients prior to antibiotic therapy.[21] The same authors also suggest normalization of the CD57+ cell numbers correlate with improvement of the patient's clinical condition. Most importantly, it was observed that the ongoing neurologic and musculoskeletal symptoms correlated particularly well with

persistently decreased levels of CD57+ lymphocytes, which return to normal when symptoms become quiescent. However, the CDC and the National Institute of Health, strongly recommend against relying upon CD57+ lymphocyte levels to direct care of patients with CLD.[22,23]

VIsE C6-Peptide ELISA

The C6 peptide represents an invariant sequence of region 6 of Variable Major Protein-like sequence Expressed (VlsE). This is a variation protein of *B. Burgdorferi* that exhibits measurable antigenic behavior. The enzyme-linked immunosorbent assay (ELISA) measures the IgG immunoglobins to the VIeE. These IgG antibodies for this invariant region develop within the first week of infection and are useful in the acute setting. The VlsE-directed ELISA may aid in the serodiagnostic evaluation of Lyme disease.[24] It is important to understand the implications of the C6 peptide ELISA as a diagnostic tool when treating patients with Lyme disease. This test may have advantages over the CDC-recommended two-tier test in terms of ease of administration, standardization, and result interpretation. If nothing else, it could be considered a valuable secondary method of diagnosis.

Studies examinging C6 peptide behavior over the clinical course of Lyme disease are scarce. Current literature suggests that VIsE C6 peptide ELISA may be a useful tool for the diagnosis of Lyme disease during the period of acute seroconversion, as standard serologic tests at this point may have negative results and for monitoring of disease progression.[25,26] Some studies imply that C6 peptide ELISA could be very sensitive for later Lyme disease stages. The antibody titers against the invariant region of C6 peptide generally decline with LD Lyme and CLD. In addition, these antibody titers do not seem to be affected by antibiotic treatment of CLD.[27]

C4a and C3a

C4a and C3a are components of the complement system which are integral to innate immunity and vital to the antibody-mediated immune response. This results in phagocytosis, lysis, and elimination of microbes.[28] Of the myriad complement proteins, C4a and C3a play a vital role in the immune response against Lyme disease. These complement proteins have reproducible behavior in response to typical antibiotic treatment for acute Lyme disease. C4a and C3a protein levels appear markedly elevated in patients who have confirmed Lyme disease, either with EM presentation or otherwise positive serological testing as defined by the CDC. Following treatment, the levels of C4a and C3a proteins return to normal ranges.[29]

C4a and C3a complement proteins follow a different pattern in the case of CLD and LD Lyme disease. Patients with LD Lyme disease have normal C3a levels but elevated C4a levels. CLD patients with predominantly musculoskeletal symptoms have very high levels of C4a. Finally, C4a levels in CLD patients with predominantly neurologic manifestations tend to remain above normal ranges. Some suggest that Lyme-directed antibiotic therapy in CLD may result in the normalization of C4a levels.[30]

Interleukin-6

Interleukin 6 (IL-6) is a cytokine produced by macrophages, B-cells and T-cells, and fibroblasts. IL-6 mediates a crucial role in regulating the immunologic response to infections, inflammations, and tissue injuries.[31] IL-6 mediates the coordination of the innate immune response to the *B. burgdorferi* spirochete infections. The systemic consequences of this response are fatigue, body aches, cognitive impairment and altered hypothalamic pituitary adrenal axis function. Patients with CLD, especially with predominantly musculoskeletal variety of the illness, have elevated IL-6 levels. Elevated IL-6 levels are due to *B. burgdorferi* spirochete infections increasing endothelial permeability, allowing the spirochete to seep pathogenically into the synovium and other joint fluids. IL-6 presence is thought contributory to the characteristic musculoskeletal symptoms in both LD Lyme disease and CLD.[32,33] IL-6-related inflammation has also been implicated in the neurological sequalae of CLD. Elevated IL-6 levels in the cerebrospinal fluid have been correlated with CNS vasculitis.[34,35]

Despite the correlations between IL-6 levels and CLD, IL-6 levels have been shown to remain elevated after antibiotic treatment.[36] This limits the usefulness of IL-6 levels to diagnose late Lyme disease. The cascade of the inflammatory events triggered by the spirochete could persist long after the disappearance of the spirochetes.

BBK07 and OppA2 Peptides

Other measurable surrogate markers in Lyme disease include the BBK07 and OppA2 peptides. Protein-based detection of recombinant BBK07 antigen utilizing line-blot assays has shown >90% sensitivity and nearly 100% specificity in diagnosing human Lyme infections.[37] OppA2 peptides also show immense diagnostic promise. OppA2 linear epitopes are contained in short peptides and are unique to *B. burgdorferi*. Therefore, OppA2 proteins maybe more specific antigenic targets for accurate diagnosis of Lyme disease. OppA2 are highly conserved in major pathogenic *Borrelia* species responsible for most Lyme diseases cases in North America and Europe. Currently, OppA2 antigens can be detected with reasonable sensitivity and specificity.[38] However, some suggest that the expression of the OppA2 protein is best documented only *in vitro*.

Luminex Multiplex Assay System

The Luminex multiplex assay system, a bead-based multiplexed immunoassay system in a microplate format, is becoming increasingly popular. This system allows for the simultaneous detection of up to 100 analytes. Ten targets were selected from sixty-two *B. burgdorferi* surface proteins and synthetic peptides were selected by assessing binding of IgG

and IgM antibody to each in a training set of Lyme disease patient samples and controls. The validation study of the 10-antigen panel identified a higher proportion of early Lyme disease patients as positive at the baseline or post-treatment visit compared to two-tiered testing (87.5% and 67.5%, respectively, $p < 0.05$). Equivalent specificities of 100% were observed in 26 healthy controls. A positive Luminex test was also associated with longer illness duration. The improved sensitivity and comparable specificity of the 10-antigen panel compared to two-tiered testing in detecting early *B. burgdorferi* suggests that multiplex analysis may improve selection of patients who may derive long-term benefit from treatment for early Lyme disease.

Treatment Guidelines

Early-stage Lyme disease is treated with curative intent. The most effective antibiotics for early-stage Lyme disease are doxycycline, amoxicillin, and cefuroxime.[39] The optimal duration of therapy in early-stage Lyme disease is 10 to 21 days. Patients with persistent symptoms, should raise consideration of coinfection with other tick-borne diseases. In early disseminated Lyme disease, studies suggest either month-long oral doxycycline or a similar course of intravenous (IV) ceftriaxone, in the absence of neurological or cardiac abnormalities. Parenteral antibiotics are advised in patients with Lyme-induced cardiac or neurologic complications.

Treatment of LD Lyme disease is more complicated. Individual variations in the rate of disease progression and the severity of the symptoms may render standard antibiotics ineffective. In a small percentage of LD Lyme patients, the disease may persist for many months or even years. These patients may experience gradual symptom resolution following oral or IV antibiotic treatments. In some cases, several courses of either oral or IV antibiotic treatments may be indicated. However, long-term IV treatment courses, longer than the recommended 4-6 weeks are not usually recommended. While some speculate that long-term courses may be more effective than the recommended 4-6 weeks, there is no current supporting scientific evidence.[40] Treatment of CLD is poorly defined and understood. Disease management is supportive and long-term antibiotics are not proven to be effective.[41]

Conclusion

Establishing diagnosis of early and late Lyme disease remains challenging. While the diagnosis of acute Lyme disease can be entirely clinical, the diagnosis of LD Lyme disease or CLD is not clinically driven. This is especially true as patients either may not recall the characteristic EM rash at presentation or suffer from other Lyme-like illnesses such as Southern Tick-Associated Rash Illness (STARI).[42] Even more difficult to interpret are the symptoms characterizing CLD. These symptoms are usually varied, atypical, and lacking in objective and measurable clinical parameters. Thus, it appears that inaccurate epidemiological details, inaccurate diagnostic methods and varied clinical parameters are woven into the fabric of CLD.

The gold standard of Lyme disease diagnosis has involved the detection of spirochete-directed antibodies. The CDC currently defines a two-tiered serologic analysis to diagnose Lyme disease. First, various antibody-mediated techniques, immunofluorescence or enzyme immunoassays are used to detect spirochete-directed antibodies. Then, for positive or equivocal antibody screen results, a Western Blot is performed to confirm the diagnosis. Despite being the *de facto* method with which to diagnose Lyme disease in the United States, there are controversies regarding the accuracy of this approach, especially in the later phases of the disease. For example, many months may pass from disease onset to the development of robust antibody titers against *B. burgdorferi.* Therefore, a prompt serologic diagnosis may not be feasible in acute Lyme disease and may be very challenging even in later phases of Lyme disease. Compounding this issue, the two-tiered Lyme disease serologic testing system currently endorsed by CDC may lack adequate sensitivity and specificity. Though the assay sensitivity appears to increase with disease progression, the assay sensitivity rarely exceeds 75% in early Lyme, and plateaus around 80% for CLD or LD stages of the disease.[43] In addition, the vast array of *Borrelia* genospecies requires the use of recombinant antigens for accurate results. The latter method, however, is both prohibitively laborious and expensive.

The shortcomings of typical two-tiered Lyme disease testing have paved the way for large numbers of alternative diagnostic techniques. However, these alternative diagnostic tests lack validation, are costly, not reproducible and may complicate the overall diagnostic picture. One exception may be the VIsE C6-peptide ELISA assay which is thought to be a useful diagnostic tool for the periods of acute and post treatment seroconversion of Lyme disease. Despite promising assays, antibody-based assays remain the only diagnostic tests approved by the US Food and Drug Administration. We recommend all Lyme disease assays be limited to patients with high probability of disease exposure.

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