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Review Article

Effective Techniques in the Definitive Diagnosis of Lyme Disease

Corey J. Coleman and Jill D. Henning*

Department of Biology, University of Pittsburgh, USA

***Corresponding author**

Jill D. Henning, 450 Schoolhouse Rd, Department of Biology, University of Pittsburgh, Johnstown, Pa, 15904, USA, Tel: 814-269-7285; Fax: 18142692022; E-mail: henning@pitt.edu

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Abstract

Lyme disease is the most prevalent vector-borne bacterial infection caused by the spirochete *Borrelia burgorferi* [1]. In the United States, transmission occurs via its tick vector *Ixodes scapularis* [2]. According to the CDC, Lyme disease is the most commonly reported vector borne illness, with its density the highest in the Northeastern United States and upper Midwest United States [3]. Because of this, proper diagnosis of this disease is imperative, however, Lyme disease is commonly misdiagnosed as another condition, or over-diagnosed in patients with non-specific symptoms [4]. This article aims to evaluate the various techniques for the diagnosis of Lyme disease to determine which standard is most efficacious for an accurate and definitive diagnosis.

SIGNS, SYMPTOMS AND IMAGING

Signs, symptoms, and imaging studies are pivotal cues in honing the differential diagnosis, and thus play a key role in differentiating Lyme disease from other maladies with common pathological features. In early Lyme disease, many signs and symptoms are non-specific and could be caused by a wide variety of etiologies, however one of the most specific and common signs of early Lyme disease is the development of erythema migrans (EM) [5]. EM can present in many fashions such as homogenous, central erythema, and central clearing, with homogenous EM being the most common [5]. However, EM-like rashes can sometimes appear with other etiologies, such as from Lone Star Tick bites [6]. Occasionally, these bites can appear indistinguishable from EM and thus possibly result in a misdiagnosis [6]. Therefore, even though EM is one of the most prevalent and specific signs of Lyme disease, non-experienced providers could potentially be misled. Furthermore, many individuals do not remember a tick bite and do not develop EM, further complicating the sole use of EM in the diagnosis of Lyme disease [7]. This illuminates the paramount role of laboratory testing in these patients.

Neurologic progression of Lyme disease, a manifestation seen in approximately 12% of patients, produces symptoms which have diagnostic relevance [3]. In a study examining the chronic neurologic manifestations of erythema migrans borreliosis, 50% of the patients experienced cranial nerve impairment, with cranial nerve VII and cranial nerve VIII palsies being the most prevalent cranial nerve manifestations [8]. Approximately 66% of the participants experienced para and tetraspasticpareses [8]. Other abnormalities such as ataxia, bladder dysfunction, and psychiatric disorders were also reported, but were not as prevalent [8]. The symptoms gain specificity as they are consolidated while individually, they can mimic a wide variety of diseases. However, the symptoms reported in this study manifested late in the progression of the disease (7 months to 12 months until diagnosis), making them generally unreliable for early Lyme disease or in patients without neurologic involvement.

It appears that children with Lyme disease also appear to have neuroimaging findings that correlate with cranial nerve disorders, according to a study by Ramgopal et al., This study evaluated neuroimages of children who presented with concurrent intracranial hypertension and Lyme disease. Of the seven total patients in the study, six received a contrast enhanced MRI of the brain and orbits, all of which showed cranial nerve involvement in at least two cranial nerves [9]. Symptoms of these patients upon presentation were also vague, with all patients presenting with a headache, four of the seven patients presenting with diplopia, six patients presenting with nausea/vomiting, and four patients presenting with photophobia [9]. This suggests that cranial nerve involvement in Lyme disease is prevalent, though symptoms related from this involvement may not always be apparent [8,9]. Though Lyme disease in conjunction with intracranial hypertension is rare, cranial nerve enhancement has been seen elsewhere in patients presenting with neurologic manifestations of Lyme disease [10,11]. In patients presenting with an array of neurologic symptoms that appear to follow a bacterial infection, particularly in patients that do not have evidence of a tick bite or EM, neuroimaging with signs of cranial nerve enhancement should draw suspicion to Lyme disease as a differential diagnosis.

Aside from clinical aspects, geographic location should not

hinder the suspicion of Lyme disease. Ticks have been shown to spread great distances by utilizing attachment to birds and thus can heavily contribute to the acquisition of Lyme disease in areas where the disease is not prevalent [12]. Because of this, geographic location should be given little consideration when adding Lyme disease as a possible differential diagnosis.

Even though Lyme disease can produce unique signs and symptoms during progression, imaging and clinical signs and symptoms do not provide a definitive diagnosis. Moreover, consideration of geographic location is an important tool in raising suspicion of Lyme disease, but cannot be used for diagnosis. In order to achieve this definitive diagnosis, laboratory testing should be performed.

CULTURING

In a study conducted by Nowakowki et al., culturing was found to be inferior in sensitivity to PCR and serologic testing of acute-phase samples [13]. Both skin cultures and blood cultures were tested, with skin cultures obtaining 51.1% sensitivity and blood cultures obtaining 44.7% sensitivity; when considered together, culturing produced 66.0% sensitivity [13]. During the study, the tissue specimen used for skin culturing was the same sample used for quantitative PCR which had 80.9% positivity [13]. Another study found that PCR was three times more sensitive than culturing from blood samples, suggesting that a large volume of spirochete must be present in order for it to be detected by culturing [14]. In an attempt to increase the sensitivity of blood cultures, Wormser et al., increased the volume of blood used for the cultures, finding that this did not increase the sensitivity [15].

Culturing alone has a sensitivity well below acceptable levels for diagnosis, however, when used in conjunction with other methods, it has the ability to enhance the overall diagnostic value [16]. Utilizing PCR, serological testing, as well as culturing has the ability to give a sensitivity of 100% [16]. However, in the clinical setting this could be problematic. PCR had a 92% sensitivity alone which could be accepted as a successful lone diagnostic technique [16]. Using 3 combined methods is also time consuming and is unnecessary for the increase in sensitivity it provides. From this data, culturing appears to be an insufficient technique for diagnosing Lyme disease.

ELISA

In a study examining the antibody response of IgM and IgG antibodies in Lyme disease, it was found that ELISA produces diagnostic levels of IgM and IgG throughout the course of the disease [17]. Using the *I. dammini* spirochete, IgM titers were highest during the ECM phase of the disease and gradually declined thereafter, while IgG titers remained high throughout the course of the disease, having at least a four-fold increase [17]. The IgG titers remained high, sometimes for years after disease onset, while IgM titers fell three to six weeks after disease onset [17].

Another study found that IgM and IgG titers for ELISA had a much lower success rate. To prepare for the ELISA, an antigen preparation of *B. burgdorferi* was utilized. During the first two weeks of the disease, two out of 22 patients had a positive IgM titer and none had a positive IgG titer [18]. Acute-phase sera and convalescent-phase sera of patients greater than or equal to 3 weeks of disease onset were also tested. When IgM and IgG responses were considered together, acute-phase sera showed a 30% positivity while convalescent-phase sera showed a 60% positivity [18]. ELISA gave false-positive results in four of the 12 patients with Rocky Mountain Spotted Fever, and in seven of the nine patients with syphilis [18]. This shows markedly different results than Craft, Grodzicki, and Steere's study, noting however that more patients were examined in this study [17,18]. This difference could also be due to the strain of the spirochete used.

A more recent study in Europe demonstrated poor sensitivity of ELISA in early Lyme disease, with a higher sensitivity in more progressed cases such as Lyme arthritis [19]. Another study, however, found that ELISA sensitivity fell below 50% in patients that have been ill for more than 4-6 weeks [20]. In the clinical setting, it can be challenging to determine the possible stage of the disease so that the best diagnostic technique can be used. It is apparent that the sensitivity of ELISA can widely vary, posing a major problem in utilizing ELISA as diagnostic technique in any stage of Lyme disease.

ELISA demonstrates a poor sensitivity early in Lyme disease, most likely because of the low amounts of antibody in serum. As the disease progresses, ELISA can become more sensitive, but even this notion is challenged by more recent evidence [18-23]. However, it is certain that ELISA poses a problem with specificity. Not only can other conditions such as Syphilis produce false-positives in ELISA, but it appears that frequent environmental exposure can also produce high false-positives [18,21]. Several different ELISAs are available, such as the Recom Well Borrelia IgG, that have increased specificity, but sensitivity is sacrificed to achieve this [22]. Therefore, ELISA alone seems to be unreliable as a diagnostic technique in early Lyme disease due to its low sensitivity, and is unreliable across all stages of Lyme disease because of its non specificity and fluctuating diagnostic accuracy. This low specificity, however, has been attempted to be corrected by coupling ELISA testing with the immunoblot, a guideline currently recommended by the CDC (discussed below) [24].

IMMUNOBLOT

Immunoblotting, like ELISA, has significant sensitivity issues in patients with early Lyme disease [25]. Dressler et al., found that ELISA using sonicated spirochetes had a 32% sensitivity and a 100% specificity for IgM early in the disease whereas IgG had a 83% sensitivity and a 95% specificity early in the disease [25]. The sensitivity of the immunoblot from IgM was increased to 44% in convalescent-phase sera [25]. This increase in sensitivity might have been caused by the parameters set in the study. At the time of the study, there was no standard to differentiate a positive immunoblot and a diagnostic immunoblot [25]. This study required 2 of the 8 most common IgM bands to be present, and 5 of the 10 most common IgG bands [25]. Without these parameters, 21% of the individuals would have met the requirements for a positive diagnostic immunoblot, and thus the sensitivity would have increased significantly [25]. In the patients with EM, these requirements lowered the sensitivity of acute-phase sera immunoblots by 7%, and lowered the sensitivity of convalescent-phase sera by 10% [25].

Another study, however, showed value in the immunoblot for early Lyme disease. Using the standard of two of three bands present for a positive IgM immunoblot and two of five bands present for a positive IgG immunoblot, the IgM had a sensitivity of 92% to 94% and the IgG showed a sensitivity of 93% to 96% [24]. The ELISA was also examined in this study and exhibited decreased sensitivity and specificity relative to IgG and IgM immunoblots [24]. This study also established a criterion to enhance the sensitivity of the immunoblot while simultaneously not greatly affecting specificity [24].

Currently, the CDC recommends a two-tier laboratory process consisting of an ELISA (or rarely, an immunofluorescence assay) and an immunoblot, both of which must be positive for a definitive diagnosis [26]. An IgG and IgM immunoblot is performed when the ELISA is positive and signs and symptoms have been present for less than 30 days [26]. In cases where the ELISA is positive and signs and symptoms have been present for greater than 30 days, and IgG immunoblot is performed [26]. Five IgG bands and two IgM bands must be present for the immunoblot to be considered positive [26]. ELISA lacks in specificity, an area where immunoblotting excels. Because of this, immunoblotting used in conjunction with ELISA would invariably enhance specificity, but the sensitivity issues would still remain [18].

The two-tier method has been recently shown to produce low sensitivity across most stages of Lyme disease with the exception of Lyme arthritis and Lyme disease with late neurologic manifestations [27]. This study, as well as the more recent research demonstrating the inadequate sensitivity of ELISA, shows that the two-tier algorithm contains serious flaws and has the possibility of missing a large number of Lyme disease cases [19,20,27].

A new immunoblot has recently been developed with a high sensitivity and specificity [28]. Utilizing two strains of *Borrelia burgdorferi* as well as adjusting diagnostic criteria to reactivity to any two of the six *Borrelia burgdorferi* antigens, the immunoblot acquired a sensitivity of 97.1% while the specificity remained greater than 93% for both IgM and IgG [28]. The study compared this data to a commercial immunoblot using CDC interpretation criteria which was found to have a sensitivity of 77.1% and a specificity greater than 97% for both IgM and IgG [28]. Not only does this further demonstrate a flaw in the current CDC recommendations, but it also shows a promising and simpler technique to definitively diagnose Lyme disease.

PCR

In a study comparing diagnostic values of various techniques in the diagnosis of early Lyme disease, quantitative PCR on skin-biopsy derived material was found to be the most sensitive producing a positivity of 80.9% [13]. Conventional-nested PCR was superior to blood culture, skin culture, and serologic testing of acute-phase samples, showing a positivity of 63.8%, but inferior to 2-stage serologic testing of convalescent-stage samples (which were 66% positive) [13]. Three of the 47 patients tested negative for all the techniques used, and thus may not have had Lyme disease [13]. If these individuals did not have Lyme disease, quantitative PCR would have an 86.4% sensitivity.From this study alone, it appears that quantitative PCR dominates other techniques for early Lyme disease. The common factor among all the patients was at least one EM lesion, from which a 2mm sample of tissue was sampled for skin-biopsy related techniques [13].

In a study conducted by Shwartz et al., quantitative PCR was generally unsuccessful in diagnosing early Lyme disease, producing 59% sensitivity to *B. burgdorferi* [29]. The most obvious variance between Shwartz's study and Nowakowski's study was the region chosen to be amplified. Shwartz et al., chose to amplify 23s rRNA genes, whereas Nowakowski's study selected to amplify the recA gene [13,29]. The 23s rRNA genes are subject to mutations in order to confer antibiotic resistance in other species; however, the resistance was generally seen with macrolide therapy [30,31]. It is possible that tetracyclines would also have the capability of inducing this type of resistance because of their interaction with the ribosome, but this is unclear [32]. Because these types of mutations were seen in other species, it is possible that it could also occur in *B. burgdorferi* and thus induce a variation to which the primers are unable to bind with full complementarity. The recA gene, however, is thought to be involved in recombination and would be thought to have a lower incidence of induced mutations caused by antibiotics, and would therefore potentially have less variation than the 23s rRNA genes [33]. However, this would have to be further evaluated.

In an attempt to increase the sensitivity and specificity of PCR, a study amplified a region that was unique to *B. burgdorferi*, but also ubiquitous within the species [34]. Clones of chromosomal 2H1 were generated and PCR amplification was performed [34]. The researchers found that as little as 0.05 pg of *B. burgdorferi* DNA was needed in order for the DNA to be amplified to detectable levels [34]. In relation to other diagnostic techniques, it would be reasonable to assume that this contributes to the relatively high sensitivity of PCR. The low quantity of DNA needed could also be valuable in diagnosing early Lyme disease, especially in the periods where an adequate antibody response has not been generated to be detected by serological methods.

The ospA gene is another possible target for DNA amplification. In a study examining PCR's sensitivity in patients with neuroborreliosis using the ospA gene, it was found that quantitative PCR was positive in all of the 3 culture-confirmed cases of neuroborreliosis, and in 5 of the 10 neuroborreliosis patients with specific antibodies in the CSF and pleocytosis [35]. After considering a further subset of patients, PCR was found to have a sensitivity of 50% in patients with neuroborreliosis [35]. This low sensitivity could have been caused by the gene selected for amplification and also the type of sample taken from the patient. CSF specimens are found to produce a lower sensitivity than that of skin samples, even in patients with neurological involvement [36]. However, if samples are obtained from EM lesions, it is possible to acquire a high sensitivity PCR with the ospA gene, suggesting that PCR sensitivity is more dependent on sample location than the primer used [37]. Samples taken from skin biopsies of EM lesions produce sensitivities that surpass that of serological testing and culturing, yet samples taken from CSF, synovial fluid, and urine produce PCR sensitivities individually that are not diagnostically valuable [13,35,37,38].

To increase the sensitivity to an even higher degree, finding

the appropriate primer to use is key. The ospA gene was thought to be a valuable gene choice because it exists in the plasmid [39]. It appears, though, that using a chromosomal gene that is conserved is more efficacious [13,34]. A study using PCR in patients with Lyme disease compared using the chromosomal p66 gene and the ospA gene in CSF and urine samples of patients with neuroborreliosis [40]. Individually, the p66 gene yielded higher sensitivity than the ospA gene, but when considered together, the sensitivity dramatically increased [40]. The efficiency of the PCR was also raised when CSF and urine sample were examined in a parallel manner [40]. The sensitivities of both genes and both samples could be considered together as the specificity of the PCR exceeded 99% [40]. This suggests that in order to increase the sensitivity of PCR, at least two different primer sets and two different samples should be used. Furthermore, using this standard also significantly increases the likelihood of PCR correctly diagnosing a Lyme disease patient who has progressed from the early stages of the disease [40].

CONCLUSION

In the early stages of Lyme disease, EM is one of the most pivotal cues in the differential diagnosis of Lyme disease. However, because many individuals do not remember a tick bite or EM fails to develop, some type of definitive diagnostic technique should be put in place. The two-tier algorithm recommended by the CDC has been shown to be ineffective in diagnosing Lyme disease. The algorithm operates under the assumption that ELISA contributes a high sensitivity to the test while the immunoblot contributes the specificity. Recent studies have shown that this assumption is not true. ELISA has a poor sensitivity in early Lyme disease and a varying sensitivity in the later stages of Lyme disease. Moreover, the two-tier algorithm has been shown to miss more than half of progressed Lyme disease cases. Though a more sensitive immunoblot has been developed, it still must be accepted by the medical community. Until this occurs, PCR seems to be the most consistent and sensitive diagnostic test.

Quantitative PCR of an EM lesion biopsy appears to be the most sensitive and specific diagnostic test in early Lyme disease, surpassing other diagnostic techniques in both sensitivity and specificity. However, PCR can be effectively employed for detecting Lyme disease in advanced stages. Previous studies have shown that utilizing two different primer sets (p66 primer and ospA primer) as well as using at least two different sample locations increase the sensitivity of PCR. This technique not only exhibits a higher sensitivity and specificity than the current two-tier algorithm recommended by the CDC, but it also has the capability of being utilized across all stages of Lyme disease.

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