Lyme disease: diagnostic issues and controversies

Expert Rev. Mol. Diagn. 15(1), 1–4 (2015)

Maria E Aguero-Rosenfeld

Author for correspondence: Department of Pathology, New York University School of Medicine, New York University Langone Medical Center and Bellevue Hospital Center, New York, NY 10016, USA
Maria.Aguero-Rosenfeld@nyumc.org

Gary P Wormser

Department of Medicine, New York Medical College, Valhalla, NY 10595, USA

The diagnosis of Lyme disease is a controversial topic. Most practitioners and scientists recognize that Lyme disease is associated with certain objective clinical manifestations supported by laboratory evidence of infection with *Borrelia burgdorferi* sensu lato (the etiologic agent). There are others, however, who believe that patients with Lyme disease may have a wide variety of entirely nonspecific symptoms without any objective clinical manifestation and that laboratory evidence of infection by *B. burgdorferi* is not required to support the diagnosis. In reality, this perspective is not evidence based and would inevitably lead to innumerable misdiagnoses, given the high frequency of medically unexplained symptoms, such as fatigue and musculoskeletal pains, in the general population. Although those espousing this viewpoint do not believe that a positive laboratory test is required, nevertheless, they often seek out and promote alternative, unapproved testing methods that frequently provide false-positive results to justify their diagnosis. Herein, we provide a brief overview of Lyme disease testing, emphasizing current usage and limitations. We also discuss the use of nonvalidated procedures and the prospects for a reduction in such testing practices in the future.

When the microbial cause of Lyme disease was discovered in the early 1980s [1], it could not have been anticipated that within a few years this antibiotic-responsive bacterial infection would be associated with social, political and medical controversy. Although it has been well established that the organism causing the disease is *Borrelia burgdorferi* sensu lato (hereafter referred to as *B. burgdorferi*), there are certain clinicians, patients and patient advocates who attribute nonspecific symptoms to Lyme disease without objective evidence of infection by this organism [2].

Undoubtedly, *B. burdorferi* is a complex spirochetal microorganism that has a predilection for different types of tissue, leading to varied clinical presentations [3]. Although direct detection methods are generally the most accurate approach for diagnosing infectious diseases, such methods for *B. burgdorferi* have had limited use, with the exception of culture and PCR on skin (erythema migrans [EM]) lesions and PCR on synovial fluid specimens. However, as with most spirochetes (corkscrew-shaped microorganisms), such as *Treponema pallidum,* the agent of syphilis, infection with *B. burgdorferi* leads to the production of antibodies. The longer the duration of infection, the more robust the antibody response is [4]. Because *B. burgdorferi* is difficult to culture *in vitro* from many tissue sites, the most widely used laboratory method to confirm infection is the detection of antibodies. Tests for detection of antibodies are not perfect because they depend on several factors, such as the duration of time it takes for detectable levels to be produced in an infected individual, which may take up to several weeks after the onset of infection [5], and the quality of the method used to detect them. The latter involves using antigens that have the desirable sensitivity and specificity to confirm the presence of antibodies that react with *B. burgdorferi*. First-generation assays developed to detect *B. burgdorferi* antibodies often lacked the most

Keywords: controversy • diagnostic issues • Lyme disease • misdiagnosis • misinformation

EXPERT REVIEWS

10.1586/14737159.2015.989837

ISSN 1473-7159

desirable performance characteristics. Many researchers have since investigated and identified antigens of diagnostic significance, including those that are expressed predominantly or exclusively *in vivo,* for inclusion in diagnostic assays [6]. In addition, as more knowledge has been gathered on the biology of this bacterium, we have come to understand the temporal sequence of development of antibodies in infected patients better.

In 1995, in an attempt to standardize and improve the specificity of Lyme disease antibody testing, the CDC, along with other agencies, recommended the use of a two-step algorithm [7]. The first step should include a sensitive assay that detects IgG and IgM antibodies, usually an enzyme immunoassay, and if reactive, then a second-step assay is run on the same sample. The second step determines the antigens recognized by the antibodies detected in the first step using separate IgG and IgM immunoblots. The second step should not be performed if the first-step enzyme immunoassay is negative. The reason is that most first-step assays generate an objective value that correlates with the intensity of the antibody reaction, as opposed to second-step immunoblots that, for the most part, are read and interpreted visually. This subjective reading and interpretation can lead to erroneous positive results if weak bands are scored as positive in samples with negative enzyme immunoassay. As in most infectious diseases diagnosed by serology, it is expected that antibodies might not be present very early in the course of infection. Therefore, two-step testing has limited sensitivity during early infection but has excellent sensitivity in patients who have been infected for several weeks [8]. Moreover, virtually everyone should be seropositive by 6 weeks. The limited sensitivity of antibody assays during early infection is not considered a problem in most cases because the diagnosis of Lyme disease at this point is confirmed clinically by the recognition of the presence of the characteristic skin lesion EM [9]. It should be emphasized that patients may not notice these skin lesions, consequently, undergoing a complete skin examination by a healthcare professional is essential. Even in those cases in which EM was not detected or was atypical, antibodies should be detectable during the convalescent phase, a few weeks after the acute phase. Despite these recognized limitations, antibody detection following the CDC guidelines performs well for patients who have objective manifestations consistent with Lyme disease other than EM [8]. As knowledge of the antigens of significance has been expanding, it is expected that future antibody assays will have greater sensitivity in early infection without a reduction in specificity. The most common cause of poor performance of serologic testing (as in other infectious diseases diagnosed by antibody testing) is their use in unselected patient populations with a low pretest probability of Lyme disease. This is a consequence of the fact that none of the serologic assays is 100% specific. For example, even if a serologic test were 99% specific and 99% sensitive, if the pretest probability was 1% in a given population, the post-test probability that a positive test is a true positive is only 50%; if the pretest probability were 0.1%, the post-test probability that a positive test is true positive would be less than 10%. Even for a Lyme disease test that was 99% specific, for every million patients tested without Lyme disease there would be 10,000 false-positive results.

A recent study conducted to assess the use of *B. burgdorferi* serology in the USA revealed that more than 3 million tests from 2.4 million patients were performed at large commercial laboratories in 2008; two-thirds of the tests were performed using the recommended two-step testing algorithm [10]. The overall cost of such testing was close to half a billion dollars. One of the conclusions of this study was that *B. burgdorferi* antibody testing is overused. Only about 12% of the tests yielded a positive result. In a recent survey on the experience of US healthcare providers with Lyme disease and other tick-borne illnesses, it was found, contrary to recommended practice, that about 75% of providers would order antibody tests for Lyme disease when a patient presents with an EM skin lesion, and only 18.7% would initiate treatment without using antibody tests [11]. Thus, this study revealed that there are knowledge gaps on the diagnosis and treatment of Lyme disease by healthcare providers in the USA. Although two-step testing has been recommended for nearly two decades, healthcare providers at large have many misconceptions on its use. Many believe that western immunoblots can be used independently of the first step; as stated above, this practice might lead to erroneous results and should be discouraged. Others interpret the presence of any band as a positive result. However, most if not all *B. burgdorferi* antigens are cross-reactive; therefore, immunoblot interpretation is dependent on the number and type of immunoreactive bands that are found. It should be further emphasized that the IgM immunoblot seropositivity is only of diagnostic use during the first month of early disease and should not be used to support the diagnosis in patients with a prolonged illness who are IgG seronegative. These misconceptions and misinformation may be reinforced by the results of testing performed at "Lyme Specialty Laboratories" (see below).

It is also important to emphasize that the humoral immune response is often long lasting, meaning that treated patients who have resolved their infection may remain seropositive for months to many years. Retesting to assess whether the patient is cured is not justified and illogical and often leads to unnecessary repeat courses of antibiotics. In patients who are known to be seropositive, it would be desirable to have another type of assay to judge whether new onset symptoms are actually due to active Lyme disease. This is also true for those who are seropositive because of a prior asymptomatic infection that had resolved. These are recognized limitations of two-stage serologic testing for Lyme disease.

Other diagnostic methods that have been used by academic researchers include culturing of *B. burgdorferi in vitro,* or the detection of its nucleic acids by PCR. Both methods have performed with acceptable levels of sensitivity on skin samples of patients with EM [12]. Culturing of samples other than skin and blood is of limited use due to the scarcity of organisms. In published studies using well-validated culture methods, the

sensitivity of culture from blood specimens (i.e., large volumes of plasma) from patients with early Lyme disease was approximately 50% [13]. A few studies have reported much higher rates of positive blood cultures using unusual and improperly validated techniques that either could not be reproduced by other investigators or for which there was convincing evidence of contamination during the technical development phase [14]. For example, another recent study showed a sensitivity of over 90% on serum samples of tested patients. With this particular technique, the detection of *B. burgdorferi* is determined by microscopy followed by DNA sequencing [15]. A subsequent study questioned the validity of those results; DNA sequences of the cultured *Borrelia* from patients were compatible with those of the control strains of *Borrelia*. Furthermore, the majority of the genetic sequences from the patient isolates aligned with Eurasian *Borrelia* sp., which have not been found to cause disease in the USA [16].

Patients having chronic, ill-defined clinical syndromes who have tested negative using validated assays for detection of antibodies to *B. burgdorferi*, seeking a diagnosis for their condition might encounter providers who use a variety of other unconventional, nonvalidated testing methods for Lyme disease besides the serum culture method discussed above. Unfortunately, several laboratories in the USA are offering these tests. Patients tested by these laboratories may be erroneously diagnosed as having Lyme disease and then may be prescribed long courses of antibiotics. Unfortunately, long-term use of antimicrobials can lead to severe side effects and even death. The nonvalidated assays being offered by such laboratories include testing for urinary antigens of *B. burgdorferi* [17], lymphocyte markers and 'in house' antibody assays often using interpretative criteria different from what has been recommended by the CDC. A recent study documented a false-positivity rate of >50% for serologic testing performed by one 'Lyme specialty' laboratory, which uses 'in-house' developed immunoblots and unconventional interpretative criteria [18]. Many of these laboratories are offering these tests as per 'customer' requests, including the reporting of 'CDC nonspecific bands' on immunoblots. Others are offering a variety of co-infection panels, including testing for pathogens that have not been proven to be transmitted by the ticks that transmit Lyme disease.

Unfortunately, until recently, 'in-house', also known as 'home brew', laboratory developed tests (LDT) have escaped the US FDA regulatory scrutiny. Because the number, complexity and use of LDTs have expanded, including their use outside of healthcare facilities, the FDA has recently initiated a process to regulate such devices as was intended when the Food Drug and Cosmetic Act was amended in 1976. The FDA has determined that without oversight, LDTs have the potential for causing harm to patients. It is anticipated that the regulatory oversight of LDTs will start during the last quarter of 2014.

Until the cause of the ailments that afflict patients seeking attention for what they believe is Lyme disease is better understood and managed, the controversy will likely continue. Meanwhile, education of healthcare providers at large on the clinical features of Lyme disease and on the appropriate use and performance of available and reliable diagnostic tests, including their limitations, should remain a priority to enhance patient care.

Financial & competing interests disclosure

GP Wormser has received research grants from Immunetics, Inc., Rarecyte, Inc., and bioMerieux SA. He owns equity in Abbott; has been an expert witness in malpractice cases involving Lyme disease; is an unpaid board member of the American Lyme Disease Foundation; has been an expert witness regarding Lyme disease in a disciplinary action for the Missouri Board of Registration for the Healing Arts; and was a consultant to Baxter for Lyme disease vaccine development. ME Aguero-Rosenfeld has provided legal consultation on litigation involving Lyme disease. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. N Engl J Med 1983;308:733-40
2. Auwaerter PG, Bakken JS, Dattwyler RJ, et al. Antiscience and ethical concerns associated with advocacy of Lyme disease. Lancet Infect Dis 2011;11:713-19
3. Steere AC. Lyme disease. N Engl J Med 2001;345:115-25
4. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis 1993;167:392-400
5. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, et al. Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J Clin Microbiol 1996;34:1-9
6. Lawrenz MB, Hardham JM, Owens RT, et al. Human antibody responses to VlsE antigenic variation protein of Borrelia burgdorferi. J Clin Microbiol 1999;37: 3997-4004
7. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morb Mortal Wkly Rep 1995;44: 590-1
8. Bacon RM, Biggerstaff BJ, Schriefer ME, et al. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE or peptide antigens of Borrelia burgdorferi compared with 2-tiered testing using whole-cell lysates. J Infect Dis 2003;187:1187-99
9. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment and prevention of Lyme disease, human granulocytic anaplasmosis and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2006;43:1089-134
10. Hinckley AF, Connally NP, Meek JI, et al. Lyme disease testing by large commercial laboratories in the United States. Clin Infect Dis 2014;59:676-81
11. Brett ME, Hinckley AF, Zielinski-Gutierrez EC, Meade PS. U.S. healthcare providers' experience with Lyme and other tick-borne diseases. Ticks Tick Borne Dis 2014;5:404-8

12. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev 2005;18:484-509
13. Wormser GP, Bittker S, Cooper D, et al. Yield of large-volume blood cultures in patients with early Lyme disease. J Infect Dis 2001;184:1070-2
14. Phillips SE, Mattman LH, Hulinska D, Moayad H. A proposal for the reliable culture of Borrelia burgdorferi from patients with chronic Lyme disease, even from those previously aggressively treated. Infection 1998;26:364-7
15. Sapi E, Pabbati N, Datar A, et al. Improved culture conditions for the growth and detection of Borrelia from human serum. Int J Med Sci 2013;10:362-76
16. Johnson BJB, Pilgard MA, Russell TM. Assessment of a new culture method to detect Borrelia species in serum of Lyme disease patients. J Clin Microbiol 2014;52: 721-4
17. Klempner MS, Schmid CH, Hu L, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. Am J Med 2001;110:217-19
18. Fallon BA, Pavlicova M, Coffino SW, Brenner C. A comparison of Lyme disease serologic test results from four laboratories in patients with persistent symptoms after antibiotic treatment. Clin Infect Dis 2014. [Epub ahead of print]