Diagnostic Testing for Lyme Disease

Takaaki Kobayashi, MD[a,*], Paul G. Auwaerter, MD[b]

KEYWORDS

• Lyme disease • Borrelia burgdorferi • Laboratory diagnosis • Serology testing

KEY POINTS

- Clinicians need to combine knowledge of Lyme disease serologic testing performance and its limitations with potential epidemiologic exposure and clinical presentation to gauge the relative usefulness of the testing results.
- Frequent misuses of serology include testing asymptomatic patients after a tick bite; reliance on testing patients with characteristic erythema migrans (EM); and using IgM immunoblot positivity for symptoms of more than 4 weeks duration.
- A modified 2-tier test strategy (MTTT) approved in 2019 by the Food and Drug Administration that uses 2 different EIAs offers faster results and better sensitivity for detecting early Lyme disease than the standard 2-tier test strategy (STTT, using immunoblots) without compromising specificity.
- In the US, a positive STTT or MTTT in appropriate clinical presentations is sufficient to diagnose most *B. burgdorferi* infections involving the nervous system or causing arthritis with joint effusions without additional fluid or tissue sampling.
- Emerging diagnostic technologies using biomarkers, which examine earlier immune responses instead of humoral immunity, may offer higher sensitivity than STTT or MTTT in early Lyme disease, though the data are still limited.

BACKGROUND

Lyme disease is the most common vector-borne infection in North America and Europe, caused by one of the 3 common pathogenic genospecies of the spirochete *Borrelia*.[1,2] *Borrelia burgdorferi sensu stricto* is transmitted solely in North America by the *Ixodes* genus of hard ticks. In Europe and Asia, Lyme disease is predominantly caused by *Borrelia afzelii* and *Borrelia garinii*. As outlined in the preceding articles,

[a] Division of Infectious Diseases, Department of Internal Medicine, University of Iowa Hospitals & Clinics, 200 Hawkins Drive, Iowa City, IA 52242, USA; [b] Sherrilyn and Ken Fisher Center for Environmental Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA
* Corresponding author.
E-mail address: takaaki-kobayashi@uiowa.edu

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B. burgdorferi infection may cause dermatologic, musculoskeletal, neurologic, and/or cardiac illnesses.

Lyme disease can be characterized as presenting within 3 forms: early localized, early disseminated, and late stages. Early localized infection presents as a single erythematous skin lesion called erythema migrans (EM) arising from the tick bite site[3]; see Franc Strle and Gary P. Wormser's article, "Early Lyme Disease (Erythema Migrans) and Its Mimics (STARI and TARI)," in this issue. However, EM may be absent in approximately 20% to 30% of cases.[4,5] If EM is absent or missed and untreated, the spirochete can subsequently disseminate, causing presentations such as multifocal EM rash, nonspecific viral-like illness, polyarthralgia, meningitis, neuropathy, or carditis. For some, late infection may occur after incubating for weeks or months. An oligoarthritis of larger joints, characteristically the knee in adults, is the most common form (see Sheila L. Arvikar and Allen C. Steere's article, "Lyme Arthritis," in this issue).

Serologic testing for Lyme disease is not recommended for asymptomatic patients after a tick bite. Even if *B. burgdorferi* has been introduced, antibody-based testing at this time will not reflect a new infection if present. Furthermore, background seropositivity of up to 5% to 10% in some parts of the US may complicate result interpretation.[6–8] Early localized Lyme disease is clinically diagnosed by the presence of EM since standard 2-tier testing (STTT) is insensitive at this early stage of infection when the humoral response has not been mounted sufficiently. Seropositivity improves as the infection continues, becoming reliable for diagnosing later clinical manifestations, including neurologic manifestations, carditis, and arthritis if an EM history is not solidly established.

Confusion regarding Lyme disease diagnosis has accompanied this infection since its discovery in 1976. Though the causative spirochete was identified in 1981 by Willy Burgdorfer, its fastidiousness and low bacterial burden in human infection meant that neither culture nor molecular techniques were easily performed or frequently positive. Instead, indirect methods reliant on antibody responses have been the dominant method for securing a laboratory diagnosis. A 2-tier approach was recommended in 1995 by CDC[9] to improve the specificity of early generation antibody testing by using immunoblots to confirm the presence of specific restricted antibody responses to *B. burgdorferi* antigens. Although serology is part of the CDC case definition which incorporates the most frequent clinical manifestations of Lyme disease, its inclusion is not intended to restrict diagnosis to seropositive cases. In one survey among practitioners in a Lyme endemic region, a majority did not rely on the CDC case definition for diagnosis.[10] Serologic testing comes with inherent limitations, making it imperative for anyone ordering these tests to sufficiently understand their utility.

Both under- and over-diagnosis of Lyme disease have been well-described.[11–15] The reasons for high misdiagnosis rates in recent years are likely multifactorial. One explanation is that Lyme disease serologic testing reports are prone to misinterpretation, such as reliance on IgM results outside of acute disease or giving undue significance to immunoblot bands not meeting criteria.[16,17] In addition, a variety of non–FDA-approved Lyme tests developed by self-designated Lyme specialty laboratories add to the confusion and often mislead clinicians as these tests are rarely clinically validated.[18] Examples of these laboratory-developed tests that are not recommended include urine antigen, quantitative CD57 lymphocyte assays, in-house criteria for the alternative interpretation of immunoblots, specialized culture techniques, and immunologic stimulation tests.[19] This article reviews the recommended laboratory diagnostics for Lyme disease (focusing on the United States) and potential developments to improve diagnosis.

THE STANDARDIZED 2-TIERED SEROLOGY TEST: STANDARD 2-TIER TESTING

In 1994, the Association of State and Territorial Public Health Laboratory Directors, CDC, FDA, the National Institutes of Health, the Council of State and Territorial Epidemiologists, and the National Committee for Clinical Laboratory Standards convened the Second National Conference on Serologic Diagnosis of Lyme Disease. The recommended methodology uses a quantitative, sensitive enzyme immunoassay (EIA) or immunofluorescence assay (IFA) as a first test. The second-tier Western blot (immunoblot) assays follow if the first tier yields positive or equivocal results based on data showing that the immunoblots improved specificity.[9] Professional society guidelines have endorsed the US Food and Drug Administration (FDA)-approved STTT to support the diagnosis of Lyme disease in patients who have objective manifestations other than acute EM.[20,21]

The schematic summarizing the features of STTT is shown in **Fig. 1**. The first tier of STTT serves as a screening test for antibodies by sensitive EIA or IFA. EIAs examine blood for the existence of IgM and IgG (together or separately) antibodies recognizing *B. burgdorferi* antigens. The initial basis for the first-tier used in FDA-approved tests was a whole-cell sonicate (WCS) of culture-grown *B. burgdorferi*. Though still used, modifications improving the accuracy of the WCS approach have been incorporated including adsorption steps to reduce cross-reacting antibodies, antibody capture techniques, fractionation of the cells, and adding synthetically produced antigens such as surface lipoprotein VlsE (variable major protein-like sequence, expressed) or C6 (invariable region 6 of VlsE) or C10 (the conserved amino-terminal portion of outer surface protein C) peptide.[22,23] Studies have demonstrated that EIAs using the C6 epitope or VlsE protein are more specific than WCS EIAs.[22–25] IFA is now rarely used as it requires skilled technical expertise, whereas automated methodologies make EIA the modern customary choice. Suppose the results of first-tier testing are less than the clinically validated threshold. In that case, the serum is reported as negative for antibodies to *B. burgdorferi* and no further testing is needed. If the result is positive or indeterminate, second-tier testing is performed proceeding to individual IgM and IgG immunoblots.

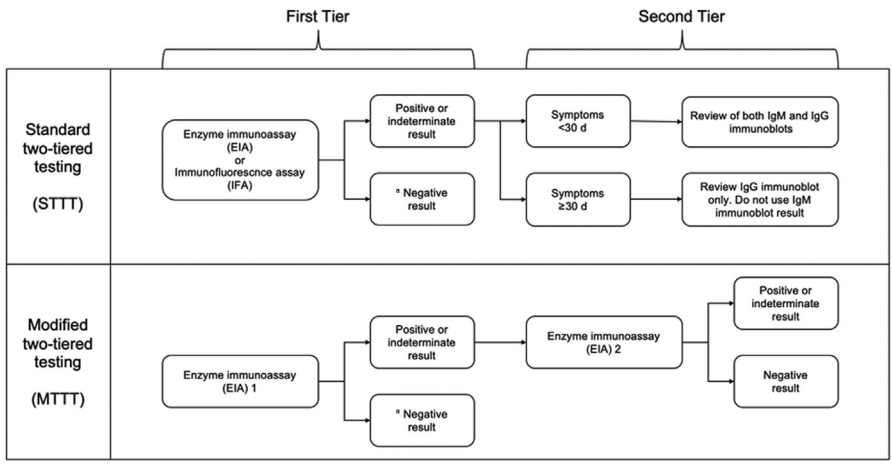

Fig. 1. Standard 2-tier and modified 2-tier tests for Lyme disease. [a] When STTT or MTTT is negative in acute disease, consider obtaining convalescent-phase serum in 4 to 6 weeks if highly suspicious for Lyme disease.

The second tier detects antibodies produced against *B. burgdorferi*, though limited to specific standardized antigens. This process uses proteins derived from *B. burgdorferi* separated in a porous gel by an electrical field. Positions of the proteins are determined by their molecular mass and then transferred to a membrane surface that is probed by serum that may contain antibodies generated to *B. burgdorferi* bacterial antigens. Such antibody–antigen binding results in detecting specific bands in the immunoblot.

The IgM immunoblot is positive if at least 2 of 3 bands (21–24, 39, and 41 kDa) stain greater than with control sera. This finding should only be used if clinical symptoms are 4 weeks or less in duration due to high rates of false positivity in patients who have longer-term symptoms.[9,26] The IgG immunoblot is considered positive if 5 or more of 10 predetermined bands (18, 21–24, 28, 30, 39, 41, 45, 58, 66, and 93 kDa) are found. These blots have been validated for *B. burgdorferi* sensu stricto for Lyme disease in North America but are less reliable for diagnosing other *Borrelia* genospecies in Europe, such as *B. afzelii and B. garinii*.[27]

Early localized Lyme disease is usually diagnosed on clinical grounds alone when a characteristic EM rash (see Franc Strle and Gary P. Wormser's article, "Early Lyme Disease (Erythema Migrans) and Its Mimics (STARI and TARI)," in this issue) is present in a patient who lives in or has recently traveled to areas with a high incidence.[21] Testing for early Lyme disease in the first week of illness may yield rates as low as 20% with STTT as humoral responses take several weeks to fully mount.[25,28,29] If there is clinical suspicion for Lyme disease, but no rash is seen or a skin lesion is atypical, and initial testing in acute illness is negative, repeating serologic testing about 3 to 4 weeks later may be helpful since the immunoblots should be positive. For later stages of Lyme disease, the sensitivity ranges from 70% to 100% with a greater than 95% specificity, including current FDA-approved testing kits[30–33] Late manifestations of Lyme disease, such as arthritis, have nearly universal IgG positive results according to 2-tier criteria, with similar seroreactivity in late neuroborreliosis.[25] Conversely, negative serologic testing among those with long-term symptoms rules out Lyme disease.[34]

THE MODIFIED 2-TIERED SEROLOGY TEST: MODIFIED 2-TIERED TESTING

FDA has recently approved a modified 2-tiered testing (MTTT) algorithm for Lyme disease serology. There is only one MTTT testing (Zeus) commercially available in the U.S as of this writing. A second EIA is used instead of an immunoblot for positive or equivocal samples on the first EIA. This may combine IgM and IgG antibodies or be performed separately. The schematic summarizing the features of MTTT is shown in **Fig. 1**. The CDC now supports MTTT as an alternative to STTT.[35] When these 2 tests are part of MTTT, specificity is greater than that of either test alone and equal to the specificity of traditional STTT.[23,36] Several recent studies comparing STTT and MTTT revealed specificity greater than 98% with MTTT.[24,25,37,38] (**Table 1**). In addition, the MTTT algorithm was more sensitive in early Lyme disease than STTT.[24,25,37,38] Similar results were reported in Canada and central Europe.[38,39] Moreover, MTTT has been shown to be more cost-effective than STTT.[33]

Although there are some features representing an improvement over STTT, limitations of MTTT remain. Similar to STTT, MTTT does not distinguish between active and past infections as antibody responses can persist for months to years. Although better than STTT for detecting early localized Lyme disease, the sensitivity of MTTT is still around 60% to 70% in early Lyme disease, which means that patients who present with EM should still be clinically diagnosed without routine serologic testing. One of

Table 1
Sensitivity and specificity of assays for the diagnosis of Lyme disease

Assay	Specimen Type	Clinical Manifestation	Sensitivity (%)	Selected References	Specificity (%)	Selected References
Standard 2-tiered testing	Serum	Early localized	< 40% (acute) 27% (convalescent) 61% (convalescent)	[32,33,97] Wormser et al,[33] 2013 Molins et al,[32] 2014	~99%	Branda et al,[36] 2017
	Serum	Early disseminated	86% (carditis) 90% 42%–87%	Molins et al,[32] 2014 Waddell et al,[98] 2016 [99]	~99%	Waddell et al,[98] 2016
	Serum	Neuroborreliosis	90%	Molins et al,[32] 2014	96%–100%	Davis et al,[39] 2020
	Serum	Late disseminated	100% (arthritis) 97%–100%	Molins et al,[32] 2014 [99]	99%–100%	Molins et al,[24] 2016, Davis et al,[39] 2020
Modified 2-tiered testing	Serum	Early localized	53% (acute) 58% (acute) 89% (convalescent) 67% (convalescent)	Branda et al,[37] 2011 Wormser et al,[25] 2013, Wormser et al,[33] 2013 Branda et al,[37] 2011 Wormser et al,[25] 2013, Wormser et al,[33] 2013	~99% 96%–100%	Branda et al,[36] 2017 Davis et al,[39] 2020
	Serum	Early disseminated	71%–86% (carditis)	Pegalajar-Jurado et al,[100] 2018	96%–100%	Davis et al,[39] 2020
	Serum	Neuroborreliosis	98%–100%	[22,37,100]	96%–100%	[22,37,39]
	Serum	Late disseminated	~100% (arthritis)	Molins et al,[24] 2016, Pegalajar-Jurado et al,[100] 2018	96%–100%	Molins et al,[24] 2016, Davis et al,[39] 2020
Polymerase chain reaction	Serum and/or skin Serum/Plasma	Early localized	64%–81% 62%	Nowakowski et al,[97] 2001 Eshoo et al,[101] 2012	~100%	Nocton et al,[102] 1994 [a], [103,104]
	Serum	Early disseminated	29% (carditis)	Molins et al,[32] 2014		
	CSF	Neuroborreliosis	25%–38% 73%	Nocton et al,[102] 1994 [a99]		
	Synovial fluid	Late disseminated	85% (arthritis) 83% (arthritis)	Nocton et al,[102] 1994 [a99]		

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Table 1
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Assay	Specimen Type	Clinical Manifestation	Sensitivity (%)	Selected References	Specificity (%)	Selected References
Culture	Skin	Early localized	51%	Nowakowski et al,[97] 2001	N/A	N/A
	Serum		45%	Nowakowski et al,[97] 2001		
	Skin + serum		44%	Molins et al,[32] 2014		
	Skin + serum	Early disseminated	0% (carditis)	Molins et al,[32] 2014		
	Serum	Neuroborreliosis	25%	Molins et al,[32] 2014		
	CSF		10%–30%	Theel et al,[105] 2019		
	Synovial fluid	Late disseminated	N/A	N/A		
CSF: serum antibody index	Serum and CSF	Neuroborreliosis	70% (<6 wk), and ~100% thereafter 82%	Koedel [76] 2015, Ljostad [77] 2007 Djukic et al,[70] 2012	97%	Blanc et al,[106] 2007

Abbreviation: CSF, cerebrospinal fluid; N/A, not applicable.
[a] Article published before 2000.

the advantages of MTTT is a shorter turnaround time, as any clinical laboratory with the capability to perform EIAs can avoid the delay incurred by immunoblot assays which take more time to perform or require a send out to a larger lab. However, for late presentations such as Lyme arthritis, STTT might be favored to distinguish the presence of specific IgG immunoblot responses (see Sheila L. Arvikar and Allen C. Steere's article, "Lyme Arthritis," in this issue).

COMMON ISSUES WITH SEROLOGIC TESTING

Serology has poor positive predictive values in areas with a low incidence of Lyme disease. One study revealed less than 20% of seropositive patients in North Carolina, an area with low incidence, had actual Lyme disease.[40] Therefore, serologic testing for Lyme disease is most useful for patients with at least an intermediate pretest probability. In addition, an EIA can be positive due to other infections or inflammatory processes. For example, both EIA and IgM immunoblots may be falsely positive due to cross-reacting antibodies to other spirochetal diseases, including relapsing fever borreliosis, syphilis, leptospirosis, and viral infections including Epstein–Barr virus, cytomegalovirus and parvovirus B19.[41–46]

Moreover, positive IgM immunoblot alone without positive IgG immunoblot is associated with overdiagnosis of Lyme disease. In a retrospective study from Boston, Lantos and colleagues[16] revealed that 30% of patients with only positive IgM immunoblots were unlikely to have Lyme disease. In the United States, the CDC recommends that a positive IgM response alone should not support Lyme disease diagnosis if symptoms have been present for more than 4 weeks since most patients will have a positive IgG immunoblot.[9] If the patient has an isolated IgM Western blot after 4 to 6 weeks from the onset of the symptoms, the IgM test is likely a false-positive result or, possibly, evidence of past infection.

Lyme disease is not an immunizing condition in early disease, and people can have second or more bouts of EM due to repeated infection.[47] However, serology for the diagnosis of subsequent infection is problematic since antibodies, including IgM and IgG, can remain positive for many years after the initial infection and successful treatment.[48] These persistent positive antibodies often render confusion due to IgM presence that could be interpreted as new or active infection requiring treatment.[48] If a subsequent extracutaneous manifestation of *B. burgdorferi* infection is suspected, it can be helpful to compare quantitative EIA values or see if additional bands are present. In these circumstances, performing acute and convalescent serologic tests would be informative to detect an increase in EIA titers or modification in the seroreactivity pattern by immunoblot.[49] However, serial testing to monitor treatment response is not recommended, although antibody responses generally decline months to years after treatment.[50,51] In addition, it is important to emphasize that immunoblots are only clinically validated for use in serum. Use in other specimens, such as cerebrospinal fluid (CSF) or synovial fluid, is not recommended as a positive result may lead to an inappropriate diagnosis of Lyme disease.[52]

Borrelia miyamotoi is a relapsing fever Borrelia group spirochete transmitted by the same hard-bodied tick species that transmit Lyme disease. *B. miyamotoi* may resemble Lyme disease but uncommonly has a rash, and frequently patients can be found to have identifiable spirochetemia, which is rare for *B. burgdorferi* infection.[53] In a study conducted in New England and New York State, the seroprevalence of *B. miyamotoi* was about one-third of *B. burgdorferi*.[6] Recent studies indicated that the C6 peptide ELISA might be positive in patients infected with *B. miyamotoi,* though do not develop *B. burgdorferi* positive immunoblots.[54] Therefore, careful interpretation

is required whereby the first-tier STTT relies solely on C6 peptide in an appropriate patient to include the consideration of *B. miyamotoi* if immunoblot negative.

A newly described spirochete, *Borrelia mayonii,* was identified in patients from Minnesota and Wisconsin with systemic symptoms and rash.[55] Currently, the diagnosis of *B. mayonii* relies on the use of a PCR test, though suspicion may also be garnered by seeing spirochetes in a Giemsa-stained blood smear. While limited studies are available on the utility of *B. burgdorferi* serologic testing on *B. mayonii* infection, available information suggests that patients with *B.mayonii* infection develop antibodies similar to those of patients with *B. burgdorferi*.[55]

CULTURE

While culture remains a standard reference method to confirm microbiological infection, it is not routinely available for diagnosing Lyme disease in most clinical settings. Culture has relatively low sensitivity, long incubation requirements (up to 12 weeks), and is technically demanding, requiring special media. Several methods can be used to confirm growth, including polymerase chain reaction (PCR) and either dark field microscopy or light microscopy using stains such as acridine orange to detect characteristic spirochetes.[56] Due to low organism burden and brief spirochetemia in early infection, culture is relatively insensitive for detecting *B. burgdorferi* in human disease.[57]

Studies have isolated *B. burgdorferi* from skin biopsy specimens, blood, and rarely CSF. Two widely used media are the modified Kelly-Pettenkofer and Barbour-Stoenner-Kelly II. The culture of skin biopsies taken from the leading border of EM has a 40% to 60% sensitivity.[58,59] One report found that blood cultures from untreated patients with EM have a sensitivity of around 45%, increasing to 70% by frequently testing culture aliquots with a sensitive PCR.[56] The sensitivity of blood culture falls to approximately 20% among those with early disseminated disease, including neurologic, cardiac or musculoskeletal manifestations.[60] Spirochetemia is principally found in patients with Lyme disease who have a relatively early infection, and *B. burgdorferi* is seldom cultured from the blood of Lyme disease patients with later disease manifestations.

The culture of CSF is rarely positive and is not recommended. CSF culture is associated with low sensitivity, ranging from 10% to 30%, in European patients with neuroborreliosis, likely lower in the relatively less neurotropic *B. burgdorferi* sensu lato genospecies causing infection in North America.[61,62] *B. burgdorferi* has not been reliably cultivated from synovial fluid, with only isolated reports of successful cultivation.[63] Finally, antibiotic treatment decreases all culture positivity rates, making it useful only in untreated patients.[64]

MOLECULAR TECHNIQUES

Though PCR technology is offered in many laboratories, it is neither an FDA-approved test nor firmly established in clinical practice. It could be pursued using a reliable laboratory backed by the clinical validation of their assay. PCR has been used in many research studies examining skin, blood, synovial fluid, and CSF. While PCR is highly specific, the sensitivity of PCR for borrelial DNA depends on the type of sample. A skin biopsy sample from the leading edge of an EM lesion has a sensitivity of 69% but a specificity of 100%.[64] In patients with Lyme arthritis, PCR of the synovial fluid has a sensitivity ranging from 46% to 96%.[65] However, the sensitivity of PCR of the CSF of patients with neurologic manifestations of Lyme disease is only 20% to 40%.[61,66] PCR of other clinical samples, including blood and urine, is not recommended, as spirochetes are primarily confined to tissues, and very few are present in these body fluids.[64]

In practice, *B. burgdorferi* DNA detection by PCR is most often used to evaluate Lyme arthritis. In patients with suspected Lyme arthritis, synovial fluid analyzed by *B. burgdorferi* PCR is highly specific but less sensitive than serum IgG seroreactivity, which is nearly always positive. Patients with characteristic knee joint effusions may not require arthrocentesis if other explanations (eg, septic arthritis, inflammatory diseases, or microcrystalline disorders) seem unlikely. Lyme arthritis in children is more likely to cause a clinically inflamed joint and fever than in adults; therefore, it can be confused with septic arthritis.[67] The disadvantage of PCR is that a positive result does not always mean active infection. The DNA of nonviable microbes may persist for several months even after successful treatment.[65] When the diagnosis of Lyme arthritis is unclear, such as in patients with a prior history of Lyme disease and existing seropositivity, PCR testing of synovial fluid may help to clarify the likelihood of active infection.

CEREBROSPINAL FLUID ANALYTICS

Common neuroborreliosis manifestations in North America and Europe are cranial neuropathies, lymphocytic meningitis, or radiculitis. Studies have demonstrated that most patients with early Lyme neuroborreliosis are seropositive by conventional 2-tiered testing at initial clinical presentation.[25,68] Lumbar puncture is not routinely required for facial palsy presentations in patients with positive serology.[21] CSF examination in Lyme neuroborreliosis typically shows a pleocytosis of more than 90% lymphocytes (mostly between 30 and 300 cells/mm^3), a slightly raised protein concentration, and a normal glucose concentration[69–71]

If CSF testing is performed in patients with suspected Lyme neuroborreliosis involving the central nervous system, it is recommended to obtain simultaneous samples of CSF and serum for determining the CSF: serum antibody index using the validated methodology in a laboratory with experience in the assay.[21] The index normalizes the level of anti-*Borrelia* antibodies to total IgG and albumin in the CSF. It establishes the antibody index ratio of anti-*Borrelia* antibodies in CSF-to-serum to suggest authentic intrathecal antibody production. Measuring the total antibody concentration only in the CSF can be misleading because a positive result may be caused by the passive transfer of antibodies from the serum. The sensitivity of the CSF: serum antibody index is considered to range from 70% to 90% in those with Lyme neuroborreliosis with less than 6 weeks of symptoms, while it is ~95% in untreated patients with longer symptom durations.[72–77] However, the index may remain elevated for years following successful treatment; therefore, careful interpretation is required.[78]

INVESTIGATIONAL TESTS

CXCL13

The chemokine CXCL13 has been proposed as an early biomarker for detecting Lyme neuroborreliosis.[79] Elevated levels of CSF CXCL13 are known to correlate with intrathecal *B. burgdorferi* antibody response in patients with acute Lyme meningitis.[80–83] However, CSF CXCL13 concentrations may be elevated in numerous conditions, including HIV infection and neoplasia. The role of CSF CXCL13 levels in CNS infection remains unestablished.[84,85]

B. Burgdorferi-Specific Cell-Mediated Responses

T cell-based assays have been the subject of research for decades to improve testing for Lyme disease, especially early infection[86]. Infection with *B. burgdorferi* elicits a T-cell response that exhibits different kinetics than the humoral response.[86,87] These tests include immunosequencing T-cell receptor (TCR) repertoires and detection of

INF-γ secretion.[88–90] Previous studies demonstrated that an active T-cell response is induced during the acute phase of infection, even in the absence of seroconversion, and returns to normal levels after antibiotic treatment and symptom resolution.[91–93] In contrast, humoral responses vary widely, demonstrating attenuated responses and a lack of IgM to IgG seroconversion that can persist for decades.[48,94] One study demonstrated a higher sensitivity with INF-γ at 69% compared with C6 ELISA at 59% and Western blotting at 17% to detect early Lyme disease.[89] In addition, a recent study from NY demonstrated that the level of INF-γ detected by the QuantiFERON ELISA significantly decreased after treatment.[95] These data may suggest that assays examining the -cellular responses may have utility for diagnosing Lyme disease, especially in an early stage, as well as evaluating the response after treatment However, each study included a small number of patients and might have included patients with other or cobacterial and viral infections concerning cross-reactivity. Moreover, controversial results and/or poor methodological quality of studies investigating T-cell response have been reported.[96] More extensive prospective studies are needed to investigate the clinical utility of immunologic cell-mediated responses for Lyme disease diagnosis.

SUMMARY

The 2-tiered serologic strategies, including STTT and MTTT, are currently the mainstays for Lyme disease diagnosis among all patients without EM. PCR of synovial fluid and CSF: serum index can be helpful in selected patients with Lyme disease. However, arthrocentesis or lumbar puncture may not be required in characteristic cases. Clinicians need to be wary of specialty laboratories offering Lyme disease testing using assays whose accuracy and clinical usefulness have not been adequately established. Moreover, clinicians should understand the clinical context of ordering serology to gauge positive and negative predictive values depending on clinical presentation, geography, and symptom duration.

Needed improvements in Lyme disease diagnostics include aiming for accurate direct pathogen-detection methods that are sufficiently sensitive and specific to detect infection by multiple pathogenic Borrelia species. Among indirect methods, serology is hampered by its inherently lagging responses in early infection. Cell-mediated immune changes may narrow the window between acquiring infection and yielding a positive test but need further study. Lastly, a future test for Lyme disease that correlates with microbiologic eradication would help staunch unnecessary antibiotic use in symptomatic people with chronic symptoms that may or may not be due to *B. burgdorferi* infection.

CLINICS CARE POINTS

- In an area with a low incidence of human *Borrelia burgdorferi* infection, positive Lyme disease test results are often false positives.
- In patients with negative serologic testing but high clinical suspicion, consider repeating serology in 3 to 4 weeks.
- MTTT has better sensitivity in early Lyme disease than STTT, with a shorter turnaround time.
- IgM results should be used only for patients with symptoms less than 4 weeks; solely relying on positive IgM responses in patients with symptoms longer than 4 weeks increases the likelihood of overdiagnosis of Lyme disease due to lack of test specificity.

- Clinicians need to be leery of insufficiently validated testing approaches such as alternative immunoblot criteria, immunoblots of CSF or synovial fluid, and testing techniques from self-described Lyme specialty laboratories.
- Synovial or CSF fluid analysis is not always required in the appropriate clinical presentations if diagnostic serum *B. burgdorferi* IgG responses are present.

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