SEROLOGIC TESTING IN THE DIAGNOSIS OF LYME BORRELIOSIS

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SUMMARY

The diagnosis of Lyme borreliosis is primarily based on clinical data. The current status of serologic testing for specific antibodies to *Borrelia burgdorferi* is reviewed. Enzyme-linked immunosorbent assay or immunofluorescence assay are the most commonly used screening tests. Western blotting can be used as a confirmatory test. Patients with early Lyme borreliosis are frequently seronegative at disease onset. Rising antibody titers in follow-up specimens can support the clinical diagnosis. In patients with neuroborreliosis, demonstration of intrathecal antibody production is a specific finding. Almost all patients with late Lyme borreliosis are seropositive, and seronegativity makes late Lyme borreliosis an unlikely diagnosis. Serologic testing for Lyme borreliosis is not standardized, and there is considerable interlaboratory variation in results. Still, with discriminate use, serologic testing can be very helpful in establishing the correct diagnosis.

KEY WORDS

Lyme borreliosis, Borrelia burgdorferi, antibodies, immunofluorescence assay, enzyme-linked immunosorbent assay, Western blot

Lyme borreliosis is a tick-borne spirochetal infection with protean clinical manifestations (1). The diagnosis is primarily based on clinical data, and clinical and laboratory criteria in the diagnosis of Lyme borreliosis have been reviewed previously (1-4). The most specific laboratory method is cultivation of the causative organism, *Borrelia burgdorferi*, with good results from skin biopsies in erythema migrans and from cerebrospinal fluid in early neuroborreliosis (5,6). However, cultures are usually negative from other tissues or blood, are not performed routinely in most laboratories, and take several weeks to grow. The polymerase chain reaction (PCR) is a powerful technique to detect bacterial DNA in human specimens, and encouraging initial results have been reported in patients with Lyme borreliosis (7,8). However, at this time PCR is limited to research laboratories and needs further investigation.

The most widely used laboratory methods have been serologic tests to detect antibodies specific for

B. burgdorferi. Following an overview of the occurrence of specific antibodies in Lyme borreliosis, the most frequently used serologic methods will be reviewed in this article.

Specific antibodies in Lyme borreliosis

Specific serum IgM antibodies to *B. burgdorferi* appear usually 3 to 4 weeks after the onset of infection, their levels peak after 6 to 8 weeks, and they subsequently decline. In a minority of patients IgM responses can be found later in the illness. Specific IgG antibodies can be detected in the serum 6 to 8 weeks after the date of infection, they may continue to rise for months or years, and the highest titers are found in patients with Lyme arthritis, chronic neuroborreliosis or acrodermatitis chronica atrophicans. Specific antibodies can also be detected in cerebrospinal fluid (CSF) and synovial fluid (9,10).

Early antibiotic treatment can abrogate the development of high antibody titers, whereas antibiotic treatment of late manifestations usually leads to only a slow decline in serum IgG titers which continue to be detectable for years. Therefore the detection of specific antibodies indicates exposure to *B. burgdorferi*, but is no proof of active infection. IgG antibodies can also persist in the CSF following therapy in the absence of further clinical symptoms (11).

Serologic tests for specific antibodies in Lyme borreliosis

Immunofluorescence assay and enzyme-linked immunosorbent assay

Shortly after the discovery of *B. burgdorferi*, the first serologic test developed was an immunofluorescence assay (IFA) (12). The organism is fixed to a microscopic slide by various methods and incubated with patients' sera and subsequently with a fluorescent anti-human antibody. The procedure is best suited for small sample volumes, the interpretation of the staining patterns is subjective and depends on the observer's experience. Using a modified IFA and living *B. burgdorferi*, better specificity was found than with a conventional IFA (13). In most laboratories the IFA has been replaced by an enzyme-linked immunosorbent assay (ELISA) which can be performed more rapidly for larger numbers of samples and has been shown to be more sensitive and specific (14-16). Most laboratories employ sonicated whole *B. burgdorferi* as antigen in microtiter plates which are read by spectrophotometer.

Due to cross-reactive epitopes both IFA and ELISA can be false positive in healthy subjects and in patients with various bacterial or viral infections, autoimmune, or neurologic diseases. False positivity is particularly common in patients with other spirochetal diseases, such as syphilis, leptospirosis or relapsing fever.

Various modifications of the standard ELISA have been introduced in order to improve sensitivity or specificity. IgM capture ELISA has been found to increase sensitivity in patients with erythema migrans (17). Preabsorption of sera with *E. coli* or *Treponema* has been reported to reduce the number of false positive results (18,19). Similarly, the use of peroxidase-labelled antigen or of purified fractions of *B. burgdorferi* including flagellin or outer-surface proteins A and B have been found to increase specificity (20-22). Neither of these modifications has been evaluated by a large number of laboratories.

More recently, recombinant preparations of some *B. burgdorferi* proteins have become available. Antibodies to recombinant outer surface proteins A and B were found in 6 of 12 patients with Lyme arthritis, but not in patients with erythema migrans. All 12 patients with Lyme arthritis and 3 of 21 patients with erythema migrans had antibodies to recombinant flagellin (23). In a comparison of an immunogenic epitope of flagellin and whole *B. burgdorferi*, sensitivity was similar while the frequency of false positive results in patients with syphilis or oral treponemal infections was reduced by about 50% (24). In a study of 37 patients with early or late Lyme borreliosis and 51 patients with no known exposure to spirochetes, the use of a recombinant 39-kDa protein of *B. burgdorferi* alone compared to soluble antigens from sonicated *B. burgdorferi* reduced sensitivity from 97% to 87% while increasing specificity from 86% to 92% (25). The best results were obtained when a 39-kDa enriched soluble sonicate preparation of *B. burgdorferi* was used with a sensitivity of 94% and a specificity of 100% in these patients (25). Recombinant antigens have not yet become widely available, but may lead to more standardized testing in the future.

Western blotting

Western or immunoblotting allows to distinguish antibody reactivity according to the molecular weights

of the antigens. *B. burgdorferi* antigens are separated by gel electrophoresis, transferred to membranes and incubated with patient sera. The technique is too labor-intensive as a screening test and has been mostly used in conjunction with IFA or ELISA. Results of Western blotting have varied. No or little additional value compared to ELISA was found in Swedish patients with erythema migrans or neuroborreliosis in whom sensitivity of blotting was somewhat higher, but specificity was low (26,27). Conversely, increased sensitivity and specificity were found in American or German patients with erythema migrans (28,29). Western blotting has been found to increase the specificity of serologic testing in patients with suspected Lyme borreliosis (30,31). Automated reading of bands with a densitometer has been advocated to establish quantitative criteria for positivity (32,33).

Interpretation of Western blots is complicated by several factors. Blotting techniques and antigen preparations vary. Cross-reactive antibodies are common, particularly against the 41-kDa flagellin or heat-shock proteins at 58- to 70-kDa (31,32).

There is no agreement about positivity criteria at this time. In a large study of American patients with various manifestations of Lyme borreliosis and controls including patients with syphilis, we found that positivity defined as the presence of at least 2 IgM bands or 5 IgG bands at particular locations gave good sensitivity and excellent specificity (31).

Laboratory variation in serologic testing for Lyme borreliosis

Significant intra- and interlaboratory variation in the results of serologic testing has been found in most studies (34-36). None of the serologic methods has been standardized. In the largest study to date, sera from 6 patients with Lyme borreliosis and 3 controls were sent to 45 laboratories. Up to 21% of laboratories failed to identify high-titer sera from patients with late Lyme borreliosis, and up to 27% identified control sera as positive (36).

Variation of antibody responses according to Borrelia burgdorferi species

The recent differentiation of three species of *B. burgdorferi* (37) has raised the question whether clinical symptoms or antibody responses vary depending on the species infecting the patient. Little is known at this point. In a French Western blot study, patients with Lyme arthritis showed preferential serum reactivity with a species 1 strain, whereas patients with neuroborreliosis showed better reactivity with a species 2 strain (38). The infecting strains were not identified in these patients. In a study of 4 patients with neuroborreliosis, no major differences were seen in ELISA responses to the sonicated antigens from the infecting strains versus heterologous strains (39).

Seronegative Lyme disease

Patients are frequently seronegative at the onset of erythema migrans or early neuroborreliosis. Later in the illness, seronegativity raises the suspicion that Lyme borreliosis is not the correct diagnosis. However, a small percentage of patients have been described who developed arthritis or peripheral neuropathy after incomplete antibiotic treatment for early Lyme disease (40,41). A cell-mediated immune response can be detected in some patients with a lymphoproliferative assay. However, the assay is labor-intensive, not standardized, and sensitivity and specificity have varied widely (40-42). Sequestration of low levels of specific antibodies to *B. burgdorferi* in immune complexes has been reported in patients who were seronegative by routine testing (43).

Detection of specific antibodies in cerebrospinal fluid

Antibodies can pass the blood-brain barrier by passive diffusion, therefore low titers of specific antibodies are not proof of intrathecal antibody production. Various methods have been used to account for differences in total protein levels in serum and CSF (9,44). Capture ELISA is an elegant method to measure the relative frequency of *B. burgdorferi* specific antibodies compared to total antibody levels, and in intrathecal production the relative amount of specific antibody is higher in CSF than in serum (9,44).

Practical guidelines for the use of serologic tests in Lyme borreliosis

Serologic testing is not needed in patients with typical erythema migrans. At the onset of erythema migrans the majority of patients have no specific antibodies to *B. burgdorferi.* In cases with atypical erythema, IgM and IgG serum antibodies should be determined, and a second sample should be tested after 4 weeks to detect rising titers. Ideally, initial

and follow-up samples should be run together. In patients with radiculopathy, cranial nerve palsies, or meningitis, serum and CSF should be examined for specific IgM and IgG antibodies. If the initial results are negative, sera should be tested again after 4 weeks. In patients with good clinical evidence of early Lyme borreliosis, treatment should be started immediately. Almost all patients with Lyme arthritis, late neuroborreliosis, or acrodermatitis have high IgG titers against *B. burgdorferi*. Absence of an IgG response strongly questions the diagnosis of late Lyme borreliosis. Patients with suspected seronegative late Lyme borreliosis should be referred to national centers to be studied further.

Serologic tests are not usually helpful in evaluating responses to antibiotic treatment. IgG titers often remain elevated for years after all clinical symptoms have disappeared following therapy.

Due to the lack of standardization of methods and the poor results in most laboratories, serologic testing should be performed in reference laboratories specialized in Lyme borreliosis. Physicians should be familiar with the methods used in their reference laboratory, and laboratory results need to be interpreted depending on the clinical data of the patient.

In the absence of clinical symptoms suggestive of Lyme borreliosis, positive results in serologic tests should not lead to a diagnosis of the disease. Positive results may be due to poor specificity of the test or previous Lyme borreliosis that has been adequately treated. Asymptomatic infection with *B. burgdorferi* has been reported with varying frequency in different areas. In patients with low positive or borderline titers by IFA or ELISA, Western blotting can be performed and help to detect false positives.

While considerable problems regarding serologic tests in Lyme borreliosis exist as highlighted in this review, with discriminate use, serologic testing can be very helpful in establishing the correct diagnosis.

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