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Recommendation To Include OspA and OspB in the New Immunoblotting Criteria for Serodiagnosis of Lyme Disease

EILEEN HILTON,[1]* JAMES DEVOTI,[1] AND SUNIL SOOD[2]

Department of Medicine[1] and Department of Pediatrics,[2] Long Island Jewish Medical Center, Long Island Campus for Albert Einstein College of Medicine, New Hyde Park, New York 11042

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In October 1994, the Second National Conference on the Serologic Diagnosis of Lyme Disease recommended a two-step approach to serological testing. The first step was the performance of an enzyme-linked immunosorbent assay (ELISA); the second step was a confirmatory immunoblot. New criteria for the interpretation of a positive immunoblot were also recommended. The committee decided to omit the 31- and 34-kDa bands (OspA and OspB, respectively) from the choice of bands considered diagnostic for a positive immunoblot. Since we had previously included these in our diagnostic criteria for Lyme disease-positive immunoblots, we reviewed data for all patients attending a Lyme disease center with positive ELISAs and immunoblot assays for Lyme disease from 1 September 1992 to 31 December 1993. The criteria for a positive Western blot (immunoblot) were the presence of 5 of 12 bands, including the 10 recommended by the conference, and the presence of the 31- and 34-kDa protein bands. Of the 136 patients evaluated, 50 were considered to have Lyme disease. Of these 50, 4 (8%) would not have met immunoblot criteria for the diagnosis if the new recommendations were used. Had the 31- and 34-kDa bands been included as part of the diagnostic requirements for immunoblot, these patients would have been included. Although overdiagnosis of Lyme disease appears to be the more frequent problem, our concern is that the exclusion of the 31- and 34-kDa protein bands from the diagnostic criteria may result in the underdiagnosis of Lyme disease by those who would rely too heavily on serological confirmation. The addition of the 31- and 34-kDa bands to those recommended for confirmatory immunoblot should be reconsidered.

In October 1994, the Second National Conference on the Serologic Diagnosis of Lyme Disease (Centers for Disease Control and Prevention/Association of State and Public Health Laboratory Directors [CDC/ASTPHLD]) recommended new criteria for the use of serological testing and interpretation of Western blots (immunoblots) in the diagnosis of Lyme disease (1).

The recommendations were to adopt a two-step approach to serological diagnosis. The first step was the use of a sensitive screening test, i.e., enzyme-linked immunosorbent assay (ELISA) or indirect fluorescent-antibody assay. If this initial test was positive or equivocal, confirmation by immunoblotting should be performed.

A consensus on the definition of a positive immunoblot was reached. An immunoglobulin G (IgG) blot is considered positive if 5 of the following 10 bands are present: 18, 24 (OspC), 28, 30, 39, 41, 45, 58, 66, and 93 kDa. An IgM blot is considered positive if two of the following three bands are present: 24 (OspC), 39, and 41 kDa.

There was agreement that the use of OspA and OspB (31- and 34-kDa) bands did not significantly add to the sensitivity or the specificity of the IgG Western blot, as these bands may develop only late in disease and other diagnostic bands would already be present when OspA and OspB antibodies appeared. Our concern was that the elimination of these bands (OspA and OspB) from the diagnostic criteria might result in excluding some cases of Lyme disease.

We reviewed the data for all patients who had positive ELISAs and immunoblot assays for Lyme disease from 1 September 1992 to 31 December 1993 in order to assess whether any patients considered to have Lyme disease by the previously used criteria would now be considered negative according to these new recommendations.

MATERIALS AND METHODS

Our diagnostic criteria included physician-diagnosed erythema migrans and/or rheumatologic or neurological manifestations of Lyme disease. In addition, a positive ELISA confirmed by an IgG Western blot was required.

ELISAs were performed by using a commercially available kit (BioWhittaker, Inc., Walkersville, Md.). As per the manufacturer's protocol, briefly, 10 μl of the controls, calibrators, and patient sera were individually pipetted into 150 μl of pretreatment reagent, mixed, and allowed to remain at room temperature for 30 min. Tubes were centrifuged to remove the IgG-specific antibody, and 25 μl of each supernatant was then added to the appropriate well of a predilution plate to which 100 μl of serum diluent was added. A 100-μl portion of diluted sera was added to the appropriate well of the antigen plate. This plate was then incubated and subsequently washed three times. Next, 100 μl of conjugate, goat anti-human IgM at a dilution of 1:100, was added to each well, and wells were incubated for 30 min. After a final wash, 100 μl of phenolphthalein monophosphate substrate was added to the plates, and the plates were incubated. Sodium phosphate tribasic was added to stop the reaction, and plates were examined with on a spectrophotometer. Results were identified as positive or negative by using a linear regression analysis program supplied by the kit manufacturer.

Immunoblots were performed by using a commercially available kit to detect human IgG (Lyme disease IgG Marblot strip test system; MARDX Diagnostics, Inc., Carlsbad, Calif.), according to the manufacturer's instructions. Briefly, patient serum is diluted 1:100 and incubated with individual Western blot strips. If *Borrelia burgdorferi*-specific antibodies are present, they will bind to the corresponding Lyme disease antigen bands. After the unbound serum is washed from the strip, the bound *B. burgdorferi*-specific antibody is reacted with alkaline phosphatase-conjugated anti-human IgG. The strip is washed and reacted with a precipitation color-developing solution to identify specific Lyme disease antigens to which the patient has antibodies. All patient sera were tested in combination with a negative, a weak-positive, and a positive control supplied with the kit. In addition, all tests included an internal positive laboratory control.

The presence of 5 of the following 12 bands was considered diagnostic of a positive Western blot: 18, 21, 28, 30, 31, 34, 39, 41, 45, 58, 66, and 93 kDa.

* Corresponding author. Mailing address: Clinical Research Division, Long Island Jewish Medical Center, 720-05 76th Ave., New Hyde Park, NY 11042. Phone: (718) 470-4411. Fax: (718) 470-9859.

RESULTS

Of the 136 patients who were evaluated for suspected Lyme disease during that time period, 50 were considered to have Lyme disease. Of these 50, 4 patients would not have met Western blot criteria for the diagnosis of Lyme disease by the new recommendations. All patients lived or vacationed in areas of endemicity. A description of these four patients follows.

Patient 1. A 69-year-old Asian male had erythema migrans in 1989 for which he was treated with 21 days of doxycycline. One year later he complained of left knee pain, and an effusion was noted on physical examination. A year later, he developed a Bell's palsy. A magnetic resonance imaging scan in 1991 showed ill-defined hyperintensities bilaterally in the pons. At that time, on physical exam, his left knee was swollen and he had a residual left facial palsy. His IgG immunoblot was positive for 18-, 34-, 41-, 45-, and 66-kDa bands, and he had a positive serum ELISA. A lumbar puncture showed a cerebrospinal fluid/serum ratio of *B. burgdorferi* antibody of 0.92 (a ratio greater than 1 is defined as positive). He was treated with 4 weeks of parenteral ceftriaxone (2 g/day) and did well, with resolution of symptoms and signs. At follow-up 2 years later, his knee was intermittently painful without objective signs of an effusion.

Patient 2. A 63-year-old white male had complained of severe abdominal muscle wall pain for 6 months. He had a history of erythema migrans treated with antibiotics by his physician. On physical examination, he had marked abdominal distention. A magnetic resonance imaging scan of his brain showed cerebral atrophy and a possible lacunar infarct. On neurological exam, he had a radiculopathy affecting his abdominal musculature. Electromyelograms and nerve conduction velocities confirmed this. His serum ELISA was positive; IgG immunoblot showed the presence of 18-, 23-, 34-, 41-, and 45-kDa bands. His cerebrospinal fluid/serum anti-*Borrelia* antibody ratio was 1.83. After 6 weeks of parenteral ceftriaxone, he showed marked improvement, with decrease in pain and resolved distention of the abdominal wall.

Patient 3. A 66-year-old white female had erythema migrans following a tick bite in 1991 and was treated for 4 weeks with an unknown oral antibiotic. In 1994, she presented with severe knee pain and an effusion. She refused arthrocentesis. Her serum ELISA was positive, and IgG immunoblot revealed bands at 28, 31, 34, 41, and 58 kDa. She was treated again with oral antibiotics but was lost to follow-up.

Patient 4. A 35-year-old white female who had a history of a tick bite acquired in an area of endemicity presented with left-knee arthritis. A knee aspiration was unsuccessful. The serum ELISA was positive, and an IgG immunoblot showed bands at 23, 30, 34, 41, and 58 kDa.

DISCUSSION

Standardization of serological testing is a needed improvement in the diagnosis of Lyme disease. The recommendations by the CDC/ASTPHLD are based on data by Dressler et al., who prospectively evaluated 225 case and control subjects. They concluded that the following bands were considered helpful in the diagnosis of Lyme disease: 18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa. OspA (31 kDa) and OspB (34 kDa) were not included in this list. The reported sensitivity and specificity of the IgG blot after first weeks of infection were 83 and 95%, respectively (2).

Differences in antibody responses of patients with Lyme borreliosis have been noted; European investigators have reported IgG bands of 22, 41, and 60 kDa in patients with meningitis and 13, 18, 21, 23, 30, 39, 60, 73, and 94 kDa in later stages of infection (7, 8). Patients in the United States, while similar in their IgM responses (21 and 41 kDa), showed reactivity to greater numbers of spirochetal polypeptides including, in addition to those above, the 28-, 31-, 34-, 45-, 58-, 66-, and 74-kDa proteins (2). In other studies from the United States, 71% of 80 patients with arthritis had strong IgG reactivity with OspA (31 kDa) or OspB (34 kDa) or both (3–5). Although there have been some reports of cross-reactivity of human spirochetes, the 31- and 34-kDa outer surface proteins appear to be more specific for Lyme borreliosis than some of the other bands recommended for confirmation (6).

In our study, the exclusion of OspA and OspB from the diagnostic criteria would have resulted in 8% of our patients diagnosed with Lyme disease failing to meet serological criteria. As more individuals are evaluated for the presence of Lyme disease (many without risk factors or clinical signs), physicians may rely heavily on serological criteria to confirm or exclude the diagnosis. There may be a tendency to view the new recommendations as absolute, though this clearly was not the intent of the consensus committee.

Currently, the most common problem is the overdiagnosis of Lyme disease, and the development of standardized criteria for the serological diagnosis of Lyme disease is a welcome improvement. However, there is concern that the exclusion of the 31-kDa (OspA) and 34-kDa (OspB) bands from the list of bands required to make the diagnosis of Lyme disease may result in underdiagnosis by clinicians who view the criteria in a strict sense. We found that that would have occurred in 8% (4 of 50) of cases at our center. We feel the addition of the 31- and 34-kDa bands to the group of bands used for confirmatory immunoblots should be reconsidered. Clinicians should, as always, exercise clinical judgment in the interpretation of any laboratory results.

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REFERENCES

1. **Centers for Disease Control and Prevention.** 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morbid. Mortal. Weekly Rep. **44:**590–591.
2. **Dressler, F., J. A. Whelan, B. N. Reinhart, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
3. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1991. Delay in the immune response to outer-surface proteins (OSP) A and B of B. burgdorferi: correlation with arthritis and treatment failure in susceptible patients with Lyme disease. Arthritis Rheum. **34:**S43.
4. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1993. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of *Borrelia burgdorferi*. Infect. Immun. **61:**2774–2779.
5. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1995. Early and late antibody responses to full-length and truncated constructs of outer surface protein A of *Borrelia burgdorferi* in Lyme disease. Infect. Immun. **63:**2228–2235.
6. **Magnarelli, L. A., J. N. Miller, J. F. Anderson, and G. R. Riviere.** 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. J. Clin. Microbiol. **28:**1276–1279.
7. **Wilske, B., V. Preac-Mursic, G. Schierz, and K. V. Busch.** 1986. Immunochemical and immunological analysis of European Borrelia burgdorferi strains. Zentralbl. Mikrobiol. **263:**92–102.
8. **Wilske, B., V. Preac-Mursic, G. Schierz, G. Liegl, and W. Gueve.** 1989. Detection of IgM and IgG antibodies to Borrelia burgdorferi using different strains as antigen. Zentralbl. Mikrobiol. **18**(Suppl.)**:**299–309.