Western Immunoblot and Flagellum Enzyme-Linked Immunosorbent Assay for Serodiagnosis of Lyme Borreliosis

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Western immunoblot with a whole-cell lysate was compared with an enzyme-linked immunosorbent assay with a purified flagellum antigen of *Borrelia burgdorferi* for serodiagnosis of Lyme borreliosis. The assays showed similar sensitivities and specificities in detecting immunoglobulin M and/or immunoglobulin G antibodies in sera from 68 patients with neuroborreliosis and 44 controls with meningitis and encephalitis or with multiple sclerosis. Flagellum enzyme-linked immunosorbent assay is more easily standardized and seems to be a more suitable diagnostic test in a routine laboratory.

Serological testing is the usual way to confirm the clinical diagnosis of Lyme borreliosis. The most commonly used tests are indirect immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA). Western blot (immunoblot) (WB) has previously been evaluated for serodiagnosis of Lyme borreliosis and found to be more sensitive than ELISA with sonicated antigen (1, 4). In the present study, WB was compared with an ELISA based on a purified flagellum antigen of *Borrelia burgdorferi*.

The first pretreatment serum and cerebrospinal fluid (CSF) samples from 68 patients with neuroborreliosis were analyzed. The neuroborreliosis diagnosis was based on clinical evidence and analysis of CSF. Sixty-two (92%) patients had pleocytosis with lymphocytic predominance in CSF (range, 6 × 10^6 to 920 × 10^6 leukocytes per liter; median, 128 × 10^6), together with signs of chronic meningitis (7, 8), or signs of radiculitis, myelitis, or cranial neuritis. Forty-two (62%) patients remembered a tick bite, twenty-eight (41%) had had an erythema migrans, and two (3%) had lymphadenosis benigna cutis. Six patients had erythema migrans and radiculitis without pleocytosis in CSF. One serum sample and one CSF sample from each of 44 patients with meningitis and encephalitis of nonborreliosis etiology or multiple sclerosis served as control samples. All samples were analyzed by ELISA, whereas only the serum samples were analyzed by WB. The flagellum ELISA and the purification of the antigen (2) have previously been described. The antigen was prepared from *B. burgdorferi* DK 1 (5). The purity of the flagellum antigen has been demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and WB (4). The antigen for WB was prepared from *B. burgdorferi* SL 42 (3), and the method for WB has previously been described (4). The immunoblots from patients and controls were coded and interpreted blinded, according to preset criteria, without knowledge of clinical data or ELISA titers. An immunoblot (immunoglobulin M [IgM] or IgG) was considered positive if it contained a band corresponding to a 41-kilodalton (kDa) flagellum protein and at least one band corresponding to low-molecular-mass proteins of 18, 21.5, or 23 kDa. These bands have previously been found to be the first to appear in immunoblots of sera from patients with Lyme borreliosis (4). An immunoblot with a single 41- or 18- to 23-kDa band was not accepted as positive since healthy controls or controls with other diseases may have such single bands (4).

Serum immunoblots from patients and controls are shown in Fig. 1. The numbers of serum samples positive by ELISA and WB are shown in Table 1. WB was significantly more sensitive than ELISA (P = 0.002, Fisher’s two-tailed exact test) in detecting IgM antibodies but not significantly more sensitive in detecting IgG antibodies. The total sensitivity for detecting IgM and/or IgG antibodies was similar for both assays (Table 1). Ten patients (15%) had negative ELISA titers in both serum and CSF. These 10 patients had had neurological symptoms for between 1 and 20 days (median, 4 days) before serum and CSF sampling. Five of them had positive IgM and/or IgG immunoblots, and *B. burgdorferi* cells were cultured from the CSF of one of these patients (3). Three of seven patient serum samples positive by IgG ELISA but negative by WB (Fig. 2) had 41-kDa bands in their IgG immunoblots, another two had 38- to 39-kDa bands, and another two had 18- to 23-kDa bands. None of three controls with positive serum IgM ELISA had positive serum IgM or IgG immunoblots, but one of them had a 41-kDa band in the serum IgM immunoblot. These controls also had negative IgG ELISA titers, but one of them had a positive borderline IgM ELISA titer in CSF. All of the three controls with positive serum IgG ELISA had positive serum IgG immunoblots. Two of these three controls also had positive serum IgG titers by ELISA. However, after correction for antibody leakage over a damaged blood-CSF barrier, no evidence of intrathecal produced antibodies was found.

The present study shows that WB is not a more sensitive assay for diagnosis of Lyme borreliosis than IgM and IgG determination by ELISA based on a purified *B. burgdorferi* flagellum antigen. The finding that sera from three patients positive by IgM ELISA and four patients positive by IgG ELISA lacked 41-kDa bands in their immunoblots is puzzling, since the patients evidently had antibodies to the purified flagellum antigen as determined by ELISA. The explanation for this could be that the native flagellum antigen contains conformational epitopes which are denatured during the preparation of antigen for WB. The use of different antigens in ELISA and WB may also have influenced the result. However, the antigen variation between flagella from different strains is probably less than the variation between outer surface proteins (unpublished data).

With clinical information at hand, the positive serum IgM ELISA titers in three controls and the positive IgM immunoblots in another four controls were retrospectively inter-
FIG. 1. Serum immunoblots of IgM (M) and IgG (G) antibodies to *B. burgdorferi* in six patients (lanes A to F) with neuroborreliosis and three controls (lanes G to I) with mumps virus meningitis, measles encephalitis, and enterovirus meningitis, respectively. The duration (in weeks [W]) of neurological symptoms in each patient is shown above the lanes. Serum optical densities measured by ELISA are shown below the lanes. Molecular mass markers (21.5 to 66.2 kDa) are indicated to the left.

FIG. 2. Serum IgM and IgG optical densities (OD), measured by ELISA, in 68 patients with neuroborreliosis and in 44 controls with meningitis, encephalitis, or multiple sclerosis. The upper limits of normal values are indicated by horizontal dotted lines. ●, Serum samples positive by WB; ○, serum samples negative by WB.
pretended as false-positives. The visual interpretation of immunoblots includes a subjective component in evaluating the intensity of different bands, and the specificity for the WB assay will to some extent depend on the person reading the immunoblots. Automatic scanning of the intensity of bands might be an alternative to visual interpretation (6). The specificity of WB can be increased by requiring several intense bands as a criterion for a positive immunoblot; but at the same time, the sensitivity is decreased. This makes the use of WB for confirmation of borderline antibody titers determined by ELISA questionable, since some of these sera show only one or two bands in the immunoblot and no single band seems to be completely specific. Patients with high antibody titers determined by ELISA almost invariably have positive immunoblots. Confirmation in these cases seems meaningless, since WB gives no further information as to whether the antibodies represent current disease or a previous clinical or subclinical infection. Since WB is more labor intensive, mainly not quantitative, and difficult to standardize for interassay variations, it seems that ELISA, especially with purified antigens, is more suitable for analyzing large numbers of serum samples in a routine laboratory. WB may still be of value as a complement to ELISA in clinically selected cases.

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LITERATURE CITED


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<tr>
<th>Duration of neurological disease (wk)</th>
<th>No. of patients</th>
<th>No. (%) of patients positive</th>
<th>ELISA IgM</th>
<th>WB IgM</th>
<th>ELISA IgG</th>
<th>WB IgG</th>
<th>ELISA IgM and/or IgG</th>
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<tr>
<td>≤2</td>
<td>27</td>
<td>10 (37)</td>
<td>16 (59)</td>
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<td>13 (48)</td>
<td>12 (44)</td>
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<td>&gt;2, ≤4</td>
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<td>7 (41)</td>
<td>14 (82)</td>
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<td>15 (88)</td>
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<td>5 (46)</td>
<td>7 (64)</td>
<td>8 (73)</td>
<td>6 (55)</td>
<td>10 (91)</td>
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<td>No. (%) positive of controls</td>
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<td>3 (7)</td>
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<td>3 (7)</td>
<td>5 (11)</td>
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