NOTES

A Decrease in the Immunoglobulin G Antibody Response against the VlsE Protein of Borrelia burgdorferi Sensu Lato Correlates with the Resolution of Clinical Signs in Antibiotic-Treated Patients with Early Lyme Disease

Antonella Marangoni,1 Vittorio Sambri,1* Silvia Accardo,1 Francesca Cavrini,2 Valeria Mondardini,3 Alessandra Moroni,1 Elisa Storni,2 and Roberto Cevenini1

Section of Microbiology-DMCSS, University of Bologna, Bologna,1 Centro di Riferimento Regionale per le Emergenze Microbiologiche, Ospedale Policlinico S. Orsola, Bologna,2 and Division of Infectious Diseases, Ospedale di Belluno, Belluno,3 Italy

Received 4 November 2005/Returned for modification 30 December 2005/Accepted 2 February 2006

The purpose of this study was to evaluate the diagnostic performance of the LIAISON Borrelia Screen (Diasorin, Saluggia, Italy), a new automated immunoassay based on the chemiluminescent technology (chemiluminescence immunoassay). To assess whether a decrease in a negative value in the anti-VlsE immunoglobulin G (IgG) antibody titer was correlated with a positive response to treatment, a group of serially collected serum samples from 67 patients with culture-confirmed erythema migrans was retrospectively studied. All the patients had been treated with antibiotics and were free of disease within 3 to 6 months of follow-up. All the 15 patients who were found to be IgG positive at the time of enrollment and who were bled at least four times during the follow-up became IgG seronegative at 2 to 6 months posttreatment. These results indicate that a decline in the anti-VlsE antibody titer coincides with effective antimicrobial therapy in patients with early localized Lyme disease.

Lyme disease, caused by the tick-borne spirochetes belonging to the species Borrelia burgdorferi sensu lato, is a multistage infection that has become the most common vector-borne disease in North America and Europe (7, 38). In Europe three different species of B. burgdorferi sensu lato pathogenic for humans (namely, B. burgdorferi sensu stricto, B. garinii, and B. afzelii) (3, 5, 36, 39, 40) are known and demonstrate both inter- and intraspecies heterogeneity (3, 41). The illness usually begins with a characteristic, expanding skin lesion, erythema migrans (EM) (26, 27, 37). The diagnosis of Lyme disease is based on clinical and laboratory findings. Serologic testing is the most commonly used corroborative laboratory method, but serology also harbors several problems. The occurrence of cross-reacting antibodies may result in false-positive findings (1, 17–19). Furthermore, patients may still be seronegative in the early stages of the infection and the humoral immune response can be diminished after the early onset of antibiotic treatment (1, 2, 28, 39). Several attempts to standardize the serological tests for Lyme disease have been done so far (10–13, 33), but considerable variations in test results are still present among different laboratories even when they use the same strategy (33). The recommendations of the Centers for Disease Control and Prevention and the German Society for Hygiene and Microbiology (DGMM) (4, 42) have relied for years on the use of a second-tier, confirmatory test for Lyme disease when the first test yielded a positive or equivocal result. More recently, a comparison of classic two-tiered testing and a VlsE (Vmp-like sequence, expressed)-based enzyme immunoassay (EIA) reported higher values of sensitivity for the latter one, which also maintained very good specificity (2). This is a new immunoassay that uses as the antigen a 26-mer synthetic peptide (the C6 peptide) based on invariable region 6 (IR6) of the VlsE lipoprotein of B. burgdorferi (43). IR6 is a highly immunogenic peptide (15) that has been shown to remain unchanged during antigenic variation and is antigenically conserved among pathogenic B. burgdorferi sensu lato strains (9, 16, 35), although it was recently showed to be structurally heterogeneous (8). Philipp et al. (32) demonstrated that a decline in the C6 antibody titer significantly correlated with a successful treatment outcome in patients with early localized or early disseminated Lyme disease. The purpose of this study was to evaluate the diagnostic performance of LIAISON Borrelia Screen (Diasorin, Saluggia, Italy), a new automated immunoassay based on chemiluminescent technology (chemiluminescence immunoassay [CLIA]). Since a recombinant VlsE antigen obtained from the PBi strain of B. garinii is used to coat magnetic particles in the immunoglobulin G (IgG) assay, our hypothesis was that this new immunoassay could be useful for the follow-up of patients with early Lyme disease. To assess whether a decrease to a negative anti-VlsE antibody titer was correlated with a positive response to treatment, a group of serially collected serum samples from patients with culture-confirmed EM was retrospectively studied.

In this study a total of 387 human serum specimens were retrospectively studied. A total of 177 serum specimens were
obtained from 67 patients (27 men and 40 women) with culture-confirmed Lyme disease. These patients were between the ages of 19 and 78 years (mean age, 42.8 years) and had EM following a tick bite. The EM diameter ranged from 3 cm to 25 cm (mean diameter, 15 cm). All the patients came from an area of endemicity in the northeast of Italy. Only 71.6% of patients (48 of 67) recalled the tick bite, but all 67 subjects had an occupational or recreational risk of exposure to *Ixodes ricinus* ticks. A skin punch biopsy specimen (diameter, 0.25 cm) was obtained from each patient when the patient entered the study and was cultivated in Barbour-Stoenner-Kelly medium modified (ATCC medium 1914) plus ciprofloxacin (0.4 μg/ml) and rifampin (40 μg/ml); the tubes were examined weekly by dark-field microscopy for motile spirochetes over a period of at least 45 days, as described previously (21). All 67 cultures of the specimens from the patients showed positivity for Lyme disease spirochetes within 1 month. At the initial clinical evaluation, each patient was bled and given specific antibiotic therapy for Lyme borreliosis. The follow-up study was performed by taking additional serum samples at 30, 60, 120, and 180 days after enrollment. In particular, 2 patients were bled five times, 17 patients were bled four times, 18 patients were bled three times, and 15 patients were bled twice; finally, 15 patients were lost to follow-up. Patients entered into the study after having had a mean duration of EM of 20 days (range, 1 to 150 days). Two hundred ten additional serum samples were obtained from the blood bank of the St. Orsola Hospital in Bologna, Italy, and 24 samples were obtained from healthy blood donors from an area of endemicity for Lyme disease in northeastern Italy (Trento). Furthermore, a panel of 40 serum samples was obtained from patients with some of the most common biological conditions that possibly result in false-positive reactivity in Lyme disease serology. All 67 cultures of *B. burgdorferi* sensu lato strains; LD (which amplifies a 16S rRNA genomic fragment common to the three genospecies); and BB, BG, and BA (each of which amplifies a species-specific 16S rRNA genomic fragment). DNA was extracted from the spirochetes for PCR as described previously (34). These primer sets generated amplification products of 276, 357, 574, 574, and 591 bp, respectively. All the PCR reagents except the primers were from the GeneAmp kit (Perkin-Elmer Cetus). A total of 50 pmol of the appropriate primer set and 25 μl of the boiled spirochete suspension were used in each 50-μl reaction mixture. All amplifications were carried out with an automatic Eppendorf Mastercycler personal DNA thermal cycler.

Anti-*Borrelia* Plus VlsE enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Lübeck, Germany) is a quantitative enzyme-linked immunosorbent based on a mixture of whole-antigen extracts of *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* and recombinant VlsE lipoprotein of *B. burgdorferi* sensu stricto. Three calibrators and positive and negative controls were used. The results were scored as negative (ratio, <1.0) or positive (ratio, ≥1.0), following the manufacturer’s instructions.

In an attempt to improve Lyme disease diagnosis in Europe, two different panels of sera were evaluated: the first consisted of samples obtained from Italian patients with culture-confirmed erythema migrans, whereas the second was composed of sera collected from blood donors and patients with infections that could interfere with Lyme disease serology. All 67 cultures obtained from the biopsy specimens were identified as *B. afzelii* by PCR, confirming previous data from our laboratory (23), namely, that in the northeast of Italy, the prevalent genospecies of *B. burgdorferi* sensu lato is *B. afzelii*. As shown in Fig. 1, 49 samples obtained at enrollment were identified to be positive by the LIAISON Borrelia Screen IgG or IgM assay (in particular, 25 serum samples were IgG positive and 42 serum samples were IgM positive), whereas the remaining 18 serum samples were both IgG and IgM seronegative. Similar results were obtained by the Anti-*Borrelia* Plus VlsE ELISA. The sensitivity of the LIAISON Borrelia Screen assay with this group of serum specimens was 73.1%, comparable to that of Anti-*Borrelia* Plus VlsE ELISA (72.6%). It is noteworthy that no sero-
conversion was observed during the follow-up of the 18 patients initially found to be IgG and IgM negative by the LIAISON Borrelia Screen assay. This could be consistent with the rapid evanescence of the antibody response to VlsE after antibiotic treatment (30–32). As a confirmation of this hypothesis, we further studied the 15 patients who were initially found to be IgG positive by the LIAISON Borrelia Screen assay and who were bled at least four times during the follow-up: all of them became IgG seronegative at 2 to 6 months posttreatment (Fig. 2). A similar effect was not observed when Anti-Borrelia Plus VlsE ELISA IgG was used: 14 of 18 patients initially IgG positive still remained reactive when they were analyzed by this EIA method during the follow-up period.

All the tests studied showed good results when the cross-react-
ing sera were tested (Table 1). In particular, the LIAISON Borrelia Screen IgG assay had a specificity of 96.7%, whereas the Anti-Borrelia Plus VlsE ELISA IgG assay had a specificity of 95.6%. The LIAISON Borrelia Screen IgM assay was shown to be 93.1% specific, whereas the specificity of the IgM EIA was 88.3%.

The findings reported in this paper confirm and expand the observations made previously both by us and by others.

First, chemiluminescent immunoassays have been evaluated in recent years (6, 24) and have been shown to be as sensitive and specific as the immunoenzymatic technique. Moreover, this method is very simple to perform and cost saving. In the present study, the diagnostic performance of a new chemiluminescent assay, LIAISON Borrelia Screen, was compared with those obtained by a commercial immunoenzymatic assay. The LIAISON Borrelia Screen IgG assay is the first immunoassay formed as well as or better than the two-tiered algorithm. The LIAISON Borrelia Screen IgG assay was shown to be a very good predictor of treatment outcome (30–32). It is noteworthy that all the Italian patients studied had early localized Lyme disease and that they promptly received antibiotic therapy. This can explain the difference of our results from those obtained by Peltomaa and coworkers (28) in a retrospective analysis of sera obtained from patients with early or late Lyme disease. Their patients showed positive anti-VlsE antibodies 8 to 15 years after treatment, but it was hypothesized that the persistence could be due to the generation of both memory T and B cells. Our hypothesis is that our patients did not develop T cells because of the prompt therapy that they received. The LIAISON Borrelia Screen IgG assay was shown to be a useful support for clinical diagnosis, since it could be used for the follow-up of Lyme disease patients. A possible disadvantage is that this IgG CLIA method does not allow the detection of IgG seroconversion in patients who scored negative before treatment. On the contrary, one major advantage of the LIAISON Borrelia Screen assay is that it can discriminate between the IgG and the IgM responses (the commercial C6 antigen-based EIA is not able to do so), and this could be very useful for clinicians. In particular, when sera drawn from patients with EM lasting less than 10 days were tested, we found that the sensitivity of IgG detection was quite low (21.2%) but that the sensitivity of IgM detection was much more acceptable (66.7%). Similar results were obtained in a previous study (22), in which the Quick ELISA C6 Borrelia assay (Immunetics, Cambridge, Mass.) was demonstrated to have a low sensitivity (33.3%) if it was used to test sera collected from Italian patients with EM.

This study was supported in part by grant from the University of Bologna (fondi RFO; ex quota 60%; 2004) to V.S. and in part by grant Centro di Riferimento Regionale per le Emergenze Microbiologiche from Regione Emilia Romagna (2004) to V.S.

We thank Danila Bassetti for providing the blood donor samples from Trento.

REFERENCES


### Table 1. Number of negative specimens detected in the panel of control sera

<table>
<thead>
<tr>
<th>Source of sera (no.) or parameter</th>
<th>No. of serum samples positive/total no. tested (%) or value of parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIAISON Borrelia Screen IgG assay</td>
</tr>
<tr>
<td>Blood donors from St. Orsola Hospital Hospital blood bank in Bologna (210)</td>
<td>205/210 (97.6)</td>
</tr>
<tr>
<td>Blood donors from blood bank in Trento (24)</td>
<td>22/24 (91.6)</td>
</tr>
<tr>
<td>ASO**-positive patients (10)</td>
<td>10/10 (100.0)</td>
</tr>
<tr>
<td>HAV* -infected patients (10)</td>
<td>10/10 (100.0)</td>
</tr>
<tr>
<td>EBV-infected patients (10)</td>
<td>9/10 (90.0)</td>
</tr>
<tr>
<td>Syphilis patients (10)</td>
<td>9/10 (90.0)</td>
</tr>
<tr>
<td>Specificity (number of negative sera/total no. of controls [%])</td>
<td>265/274 (96.7)</td>
</tr>
</tbody>
</table>

**The results were obtained by testing the sera once.

a ASO, streptolysin O antibody titer >400 IU/ml.

b HAV, hepatitis A virus acute infection (IgM positive).

c EBV, Epstein-Barr virus (Paul-Bunne-Davidsohn agglutination assay positive).


