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

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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) are known and demonstrate both interspecies 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 (DGHM) (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 patients were enrolled. In particular, 2 patients were bled five times, and rifampin (40 µg/ml) was added. The light signal, and, hence, the amount of isoluminol-reduced. The light signal, and, hence, the amount of isoluminol-derivatives (isoluminol-antigen conjugate) in the IgG and IgM phases and to the antigen conjugate. The unbound material is removed with a wash cycle. Subsequently, the starter reagents were added and a flash chemiluminescence reaction is thus induced. The light signal, and, hence, the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units and is indicative of the concentration of antibodies to *B. burgdorferi* present in calibrators, samples, or controls. The global results of the IgM test are reported in index units and are evaluated by using a cutoff value of 1.0 index unit, with a gray zone of ±10%. The results are interpreted as follows: samples scored with an index value <0.9 are considered negative; samples with an index value ≥1.1 are considered positive; and finally, samples with a value that falls in the gray range (i.e., 0.9 < index value <1.1) are boundary cases and, following the manufacturer’s instructions, were tested again. The IgG concentration was expressed, instead, as arbitrary units (AU)/ml; the measurement range was from 0 to 240 AU/ml. Sample results were interpreted as follows: samples with IgG concentrations below 10 AU/ml were graded negative; and samples with IgG concentrations ranging from 10 to 15 AU/ml were graded equivocal, and, following the manufacturer’s instructions, were tested again. Finally, samples with IgG concentrations equal to or greater than 15 AU/ml were graded positive.

PCR was performed by using five different sets of primers whose sequences have been obtained from the literature (25, 29): FL6-FL7 (which amplifies a fragment of the flagellin gene that is conserved in all *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 assay 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 pa-
tients 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 hypoth-
esis, 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-

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**FIG. 1.** Results obtained by LIAISON Borrelia Screen assay when the 67 serum specimens drawn at the enrollment were tested. The x axis shows the duration of EM at the time that the patients entered the study. The number of samples is indicated by the number above each individual bar in the graph.

**FIG. 2.** Decrease in IgG response observed in the 15 patients studied during the follow-up, as detected by LIAISON Borrelia Screen IgG assay. Samples with IgG concentrations below 10 AU/ml were negative, samples with IgG concentrations ranging from 10 to 15 AU/ml were equivocal (as indicated by the horizontal bar), and samples with IgG concentrations equal to or greater than 15 AU/ml were positive.
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 was shown to be a very good alternative to the traditional EIAs, since it was shown to be at least as sensitive as the Anti-Borrelia Plus VlsE ELISA when samples from patients with EM were tested. Moreover, the LIAISON Borrelia Screen was slightly more specific than the Anti-Borrelia Plus VlsE ELISA; the most problematic sera for both methods seemed to be the samples obtained from mononucleosis patients, whereas EIA IgM showed a poor value of specificity when sera from blood donors from Trento, Italy, were tested.

Second, when the C6 peptide was used in a diagnostic EIA with serum samples from U.S. patients, the assay performed with a good sensitivity and a good specificity (15, 20, 28). In Europe, a few papers on the diagnostic use of the C6 peptide have been published (9, 14, 16, 22, 23). Moreover, Bacon and coworkers (2) reported that the test with the C6 peptide performed as well or better than the two-tiered algorithm. The LIAISON Borrelia Screen IgG assay is the first immunoassay based exclusively on the whole recombinant form of the VlsE antigen. In this study all sera from patients initially found to be IgG positive became nonreactive 2 to 6 months after treatment. This test confirmed what Philipp and coworkers had previously reported, i.e., that the C6 test performed well as a 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.

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**TABLE 1. Number of negative specimens detected in the panel of control sera**

| Source of sera (no.) or parameter | LIAISON Borrelia Screen IgG assay | LIAISON Borrelia Screen IgM assay | Anti-Borrelia Plus VlsE EIA IgG assay | Anti-Borrelia Plus VlsE EIA IgM assay |
|----------------------------------|----------------------------------|----------------------------------|-------------------------------------|-------------------------------------|
| Blood donors from St. Orsola Hospital & Hospital blood bank in Bologna (210) | 205/210 (97.6) | 198/210 (94.3) | 204/210 (97.1) | 188/210 (89.5) |
| Blood donors from blood bank in Trento (24) | 22/24 (91.6) | 21/24 (87.5) | 22/24 (91.6) | 18/24 (75.0) |
| ASO-positive patients (10) | 10/10 (100.0) | 9/10 (90.0) | 10/10 (100.0) | 8/10 (80.0) |
| HAV-infected patients (10) | 10/10 (100.0) | 10/10 (100.0) | 10/10 (100.0) | 10/10 (100.0) |
| EBV-infected patients (10) | 9/10 (90.0) | 7/10 (70.0) | 7/10 (70.0) | 8/10 (80.0) |
| Syphilis patients (10) | 9/10 (90.0) | 10/10 (100.0) | 9/10 (90.0) | 10/10 (100.0) |
| Specificity (number of negative sera/total no. of controls [%]) | 265/274 (96.7) | 255/274 (93.1) | 262/274 (95.6) | 242/274 (88.3) |

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

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

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

*d EBV, Epstein-Barr virus (Paul-Bunnel-Davidsohn agglutination assay positive).
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