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# Correlation of Seroreactivity with Response to Antibiotics in Pediatric Lyme Borreliosis

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**Response to treatment with antibiotics was compared with serologic reactivity and clinical symptoms in a pediatric population with presumptive diagnoses of Lyme borreliosis. The population analyzed for this study consisted of a subset of a larger Lyme clinic population being monitored as part of a prospective study on pediatric Lyme borreliosis. All patients resided in an area in which *Ixodes scapularis* and *Borrelia burgdorferi* are considered endemic. Serum from patients was tested by enzyme-linked immunosorbent assay and Western blotting. Response to antibiotics was evaluated by members of a pediatric Lyme clinic. Results showed that positive serologic test results correlate with a favorable response to antibiotics, as does the presence of erythema migrans (EM), regardless of serologic status. Seronegative patients without EM had chronic fatigue and arthralgia and/or myalgia as primary symptoms and did not respond to antibiotics, even when multiple courses of treatment were given. These results indicate that serologic tests designed to have high specificity can reliably rule out Lyme borreliosis in patients with chronic symptoms, thus preventing unnecessary treatment with antibiotics.**

Since its original description as an infectious arthritis, the clinical spectrum of Lyme disease has expanded to include a wide variety of symptoms; in addition, it is known that infection with *Borrelia burgdorferi* can affect several different organ systems (20). Positive diagnosis of infection with *B. burgdorferi* sensu stricto or one of the other currently recognized genomic species of the spirochete which causes Lyme borreliosis (LB) continues to be controversial (2, 3, 5, 14). Major areas of controversy include the reliability of serologic tests; the adequacy of standard treatment regimens, particularly in relation to chronic LB; and the diagnostic specificity of symptoms other than erythema migrans (EM) in chronic LB (4, 11). This has led to the presumptive diagnosis and treatment of LB, regardless of serologic results, for patients with chronic fatigue and chronic musculoskeletal complaints. Furthermore, the failure of such treatments is associated with retreatment or the chronic use of antibiotics. Our impression is that most physicians, particularly those involved in research, have the intuition that most of these patients do not have LB; however, little research which documents this belief has been done or published, and a substantial contingent contends that chronic LB is greatly underdiagnosed due to the insensitivity of serologic tests. The controversy has received national and international attention, as evidenced by the plans of the National Institutes of Health to initiate and fund studies of chronic LB (13).

This investigation was initiated to determine the relationship between serologic diagnostic criteria and clinical response to antibiotic therapy. The design of the investigation included a provision for the prospective inclusion of patients already being seen as part of ongoing investigations into various aspects of LB. In addition, different serologic assays were evaluated, including an in-house-developed enzyme-linked immunosorbent assay (ELISA) and Western blot, which were adopted as our standard assays for diagnostic use (7, 15, 16). A total of 146 patients at our pediatric Lyme disease clinic were included based on the availability of clinical, serologic, and treatment response data. As stratifying variables we used two independent measures: response to therapy and seropositivity. Patients were divided into two groups, one composed of those responding to antibiotic treatment and one whose symptoms persisted following treatments with antibiotics. Clinical findings and seroreactivity were then compared for the two groups.

## MATERIALS AND METHODS

**Patient selection.** The study population was comprised of 146 pediatric patients seen at a Lyme disease clinic who had received at least one course of antibiotics for suspected Lyme disease and for whom in-house serologic (Western blot and ELISA) data were available. The majority of patients were initially diagnosed prior to referral to our clinic, and all patients were monitored for at least 6 months after the end of antibiotic treatment. The patients were grouped according to their response to treatment with antibiotics. One group was comprised of 95 children considered responders (no clinical symptoms after 12 weeks), and the other was comprised of 51 children whose symptoms persisted beyond 12 weeks after antibiotic treatment. Each patient's symptoms and clinical history were documented and/or reviewed by a pediatric rheumatologist with expertise in the diagnosis of LB. Clinical categories were established by commonly accepted diagnostic criteria. Briefly, definitions were as follows: EM, physician-confirmed and/or parent-observed erythematous expanding flat skin lesion with a 5-cm minimum diameter; aseptic meningitis, acute episodes of headache and neck stiffness with a documented increase in protein concentration and cell count in cerebrospinal fluid and an absence of microorganisms in culture; Bell's palsy, acute facial nerve palsy; arthritis, synovial swelling documented by physician; rash, any inflammatory exanthem; peripheral neuropathy, motor or sensory deficit observed on a peripheral nerve territory; arthromyalgia, pain referred to joints and/or muscles in the absence of objective evidence of inflammation; fatigue, perception of inability to perform physical or intellectual tasks of which the individual was capable in the premorbid state.

**ELISA analysis.** Harvested spirochetes were sonicated in phosphate-buffered saline (PBS) on ice. A supernatant fraction was collected after centrifugation, and the protein concentration was adjusted to 5 μg/ml. Microtiter wells were incubated with this antigen solution for 2 h at 37°C, then methanol fixed for 10 min, and blocked with 0.5% bovine serum albumin in PBS. Patient serum was diluted 1:80 in *Escherichia coli* sonicate as described by Fawcett et al. (7). Following a 30-min incubation at 37°C, the wells were washed and 100 μl of a 1:1,000 dilution of peroxidase-conjugated goat anti-human immunoglobulin G (IgG) antiserum was added for 30 min at 37°C. The wells were then washed with PBS, and 100 μl of ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate(6)])

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TABLE 1. Clinical presentation for responders and nonresponders

| Patient category | *n* | Mean age (yr) | % of patients with symptom at time of first presentation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | EM | Arthritis | Bell's palsy | Peripheral neuropathy | Aseptic rash | Meningitis | Arthromyalgia | Fatigue[a] | Other |
| Responder | 95 | 8.6 | 39 | 27 | 11 | 5 | 6 | 2 | 0 | 10 | 0 |
| Nonresponder | 51 | 9.8 | 0 | 6 | 6 | 0 | 0 | 0 | 20 | 61 | 7 |

[a] The mean duration of fatigue for responders was 4.5 weeks, and that for nonresponders was 46 weeks.

was added for 10 min at room temperature, followed by the addition of 50 μl of stop solution (250 mM oxalic acid). The wells were then read at 405 nm with a Titertek Multiscan (Flow Laboratories, Helsinki, Finland).

**Western blot analysis.** *B. burgdorferi* (ATCC B31), grown to late log phase at 33°C in BSK-H medium (Sigma, St. Louis, Mo.), was used as a Lyme antigen source. Spirochetes were electrophoresed on 10% polyacrylamide gels by using the discontinuous buffer system of Laemmli and Fayre (12). Separated proteins were transferred to nitrocellulose by means of a Nova blot semidry transfer system (Pharmacia, Piscataway, N.J.). Following transfer, the nitrocellulose membranes were blocked by overnight incubation at 4°C with 0.5% bovine serum albumin in PBS. Membranes were next cut into strips, dried, and stored desiccated until needed.

Sera were tested for IgG and IgM antibodies to *B. burgdorferi* antigens by incubating rehydrated strips with a 1:100 dilution of test serum in 1% nonfat milk containing PBS for 1 h at room temperature. The strips were then washed three times and a 1:1,000 dilution of biotinylated antiserum (goat anti-human IgG γ chain specific or IgM μ chain specific) was added for a 1-h incubation; incubation was followed by three washes. Peroxidase-conjugated streptavidin (diluted 1:1,000) was then added, and the mixture was allowed to incubate for 1 h at room temperature. The strips were then washed as before and 2 ml of substrate solution (4-chloro-1-naphthol) was added. After a final wash in distilled water, the strips were dried and evaluated for reactivity by comparison with controls.

**Statistical analysis.** Data were analyzed by Student's *t* test or chi-square analysis (with Yates' correction in effect) where appropriate.

## RESULTS

Shown in Table 1 are the mean ages (not significantly different) and primary symptoms at the time of initial diagnosis for responder and nonresponder patients. Fatigue and chronic pain (mostly classified as arthromyalgia) were symptoms for over 80% of the nonresponder population but only 10% of the responders. The three most common symptoms among responders were EM, arthritis, and Bell's palsy, in descending order of frequency. No EM was documented for nonresponders, and only 6% of the nonresponders had arthritis as a primary symptom.

Figure 1 shows the percentages of seronegative and seropositive patients whose symptoms resolved within 12 weeks of receiving antibiotics. Resolution of symptoms was significantly more frequent in seropositive than in seronegative patients ($P < 0.001$) who received antibiotics. Over 90% of the seropositive (by Western blotting) patients who received antibiotics were classified as responders, while only 47% of the seronegative patients had resolution of symptoms in that time frame. The difference between the responsiveness of seropositive and seronegative groups was even more dramatic when patients with EM who were seronegative were excluded from the seronegative group; responsiveness then dropped to 28%. It should also be noted that all of the patients with EM were classified as responders.

The results of serologic tests for responders and nonresponders are shown in Tables 2 and 3, respectively. Patients in each category were separated according to primary symptoms and final diagnosis (Lyme disease or other). Fifty-seven percent of the responders (Table 2) tested positive by ELISA, and 42% were positive by Western blotting. Responders with arthritis had the highest positivity rate (100%). Furthermore, all of the EM and arthritis patients in the responder group had a final diagnosis of LB.

The results of serologic tests for the nonresponder group (Table 3) differed dramatically from those obtained for responders. Only 10% of the nonresponders were positive by ELISA and 8% ($n = 4$) were positive by Western blotting. Four nonresponder patients had a final diagnosis of Lyme disease (these patients also had positive Western blots). Two of the four had had clinically documented, treated Lyme disease 2 years prior to enrollment in this study and continued to have symptoms (chronic pain and arthritis). The other two patients diagnosed with Lyme disease in the nonresponder group had symptoms which failed to resolve within 12 weeks of antibiotic treatment. Following retreatment, symptoms resolved for both.

Table 4 shows data for antibiotic treatment of responders and nonresponders. While no difference in the likelihood of receiving oral versus intravenous (i.v.) antibiotics among the groups was noted, nonresponders were significantly more likely to have received multiple oral or i.v. courses of treatment than were responders. With the exception of two patients who were diagnosed with Lyme disease, the nonresponders did not show resolution of symptoms as a result of multiple courses of treatment. Two patients classed as responders had received a second course of oral antibiotics during the 12-week period starting with their first treatment course.

## DISCUSSION

It is well established that infection with *B. burgdorferi* can affect multiple organs and systems, effecting the plethora of clinical symptoms associated with LB (1, 9). The spirochete also has antigens which possess determinants capable of bind-

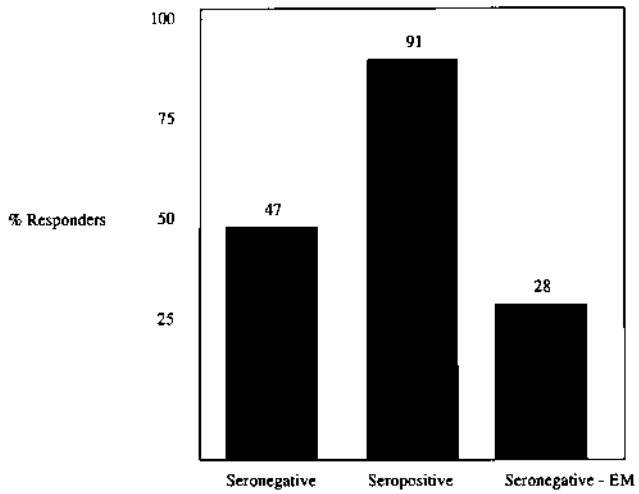


FIG. 1. Percentages of patients testing as seronegative ($n = 87$), seropositive ($n = 59$), and seronegative without symptoms of EM ($n = 64$) who were classified as responders to treatment with antibiotics.

TABLE 2. Symptoms, serology, and LB status of responders

| Symptom | *n* | No. positive by: | | | | No. with diagnosis of: | |
|---|---|---|---|---|---|---|---|
| | | ELISA[a] | Western blotting | | | Lyme disease | Other |
| | | | IgG | IgM | IgG and IgM combined | | |
| Acute arthritis | 3 | 2 | 0 | 0 | 0 | 2 | 1 |
| Peripheral neuropathy | 5 | 3 | 2 | 0 | 0 | 3 | 2 |
| Aseptic meningitis | 2 | 2 | 2 | 1 | 0 | 2 | 0 |
| Unspecific rash | 6 | 1 | 0 | 0 | 0 | 0 | 6 |
| EM | 34 | 11 | 1 | 2 | 4[b] | 34 | 0 |
| Arthritis | 26 | 26 | 26 | 0 | 0 | 26 | 0 |
| Bell's palsy | 10 | 4 | 1 | 0 | 0 | 5 | 5 |
| Fatigue | 9 | 5 | 3 | 0 | 0 | 5 | 4 |

[a] Detected only IgG antibodies.
[b] Requires two bands present at the same molecular weight for both IgG and IgM Western blots.

TABLE 3. Symptoms, serology, and LB status of nonresponders

| Symptom | *n* | No. positive by: | | | | No. with diagnosis of: | |
|---|---|---|---|---|---|---|---|
| | | ELISA | Western blotting | | | Lyme disease | Other |
| | | | IgG | IgM | IgG and IgM combined | | |
| Fatigue | 31 | 0 | 0 | 0 | 0 | 0 | 31 |
| Chronic pain (arthromyalgia) | 10 | 2 | 1 | 0 | 0 | 1[a] | 9 |
| Bell's palsy | 3 | 0 | 1 | 0 | 0 | 1[b] | 2 |
| Arthritis | 3 | 2 | 1 | 0 | 0 | 1[a] | 2 |
| Other | 4 | 1 | 1 | 0 | 0 | 1[b] | 3 |

[a] Patient reported chronic pain and joint stiffness for over 2 years after initial diagnosis and treatment.
[b] Patient symptoms persisted for over 12 weeks following initial treatment. The patient responded to a second course of treatment with antibiotics.

ing antibodies from patients with a variety of infectious and autoimmune diseases, such as Epstein-Barr virus, whose symptoms overlap those of LB (6, 7, 18). Taken together, the variability of symptoms and cross-reactive antibodies have led to LB becoming a diagnosis by exclusion in some regions, contributing to overdiagnosis and overtreatment (9, 15, 21).

The present study was initiated to determine if response to antibiotics relates to seropositivity and to determine whether LB is being overtreated in our hospital's service area, in which LB is endemic. The population studied included patients referred to our clinic subsequent to diagnosis and patients diagnosed and monitored throughout treatment at our clinic. Patients were stratified into groups based on serology (in-house-developed Western blot assay and ELISA using *E. coli* adsorption) and response to treatment with antibiotics (6, 7, 16). A final diagnosis was made based on evaluation by a pediatric rheumatologist with expertise in Lyme disease, a review of medical history, and a follow-up evaluation. The initial presumptive diagnosis of LB was ruled out for 65 of the 146 patients (44.5%) evaluated for this study.

Stratifying the population by treatment response and serology revealed a correlation between these two parameters. We next compared clinical symptoms in relation to seroreactivity. The only major difference between the populations classified by serology versus those classified by response to treatment involved patients with EM as their primary or sole symptom of infection. Such a finding was not unexpected, as patients who early postinfection may not have produced a sufficient variety or quantity of *B. burgdorferi*-specific antibodies to yield a positive serologic test respond well to treatment with antibiotics (15, 18). Indeed, in this study, 100% of patients with EM fell into the responder group, in which they accounted for 39% of the group population. Of the responder patients with symptoms other than EM, the most common symptom was arthritis, followed by Bell's palsy and fatigue. The fatigue reported for the responder group differed in duration from that observed in the nonresponder group. The mean duration of fatigue for responders was 4.5 weeks; that for nonresponders was 46 weeks. None of the nonresponders experiencing fatigue were seropositive by ELISA or Western blotting ($n = 31$), while five and three responders with fatigue were positive by ELISA and Western blotting, respectively ($n = 9$). On final diagnosis, five of the responders with fatigue were classified as having Lyme disease.

Four patients in the nonresponder group were seropositive by Western blotting and received a final diagnosis with Lyme disease. Two of these patients had been diagnosed with Lyme disease 2 years previously; of these, one had persistent chronic pain following treatment and one had chronic arthritis. The other two seropositive nonresponders, one with Bell's palsy and one reporting headaches, had symptoms which persisted for 12 weeks after diagnosis and treatment, but the symptoms resolved following a second course of treatment. Thus, all but two seropositive patients diagnosed with Lyme disease recovered following treatment with antibiotics.

The two most common symptoms for patients in the nonresponder category were fatigue and chronic pain classified as arthromyalgia. These two symptoms accounted for the symptoms of 80% of the nonresponder population, a result similar to those in previous reports (10, 15). When antibiotic regimens were compared for responder and nonresponder groups, there was no overall difference noted in the frequency of oral i.v. treatment; however, multiple courses of antibiotics were most common in nonresponders with fatigue. After final evaluation, all of the seropositive responders were classified as having definite LB, while none of the seronegative nonresponders were so classified. It is important to note also that 19% of the responders were also classified as not having Lyme disease. This is hardly unexpected, as many infections respond to antibiotics and even viral illnesses are likely to improve in the 12-week time frame defining treatment response for this study. Our data provide a strong indication that seropositivity is associated not only with LB but also with response to treatment. This is most likely due to the fact that seronegative patients with long-term symptoms do not have LB. The only observed failures of seropositive LB patients to respond to treatment occurred for two patients with Lyme arthritis of prolonged duration, a condition previously reported by Schoen et al. (17) and Steere et al. (19).

The results obtained in this study concur with previous papers reporting that chronic fatigue and fibromyalgia are fre-

TABLE 4. Comparison of treatment courses for responders and nonresponders

| Antibiotic treatment | % of patients receiving treatment | |
|---|---|---|
| | Responders | Nonresponders |
| Single oral | 65 | 53 |
| Multiple oral | 2 | 18 |
| Single i.v. | 33 | 13 |
| Multiple i.v. | 0 | 16 |

quently misdiagnosed as chronic LB. Unlike those previous reports, however, our findings demonstrate that serology can be a very effective adjunct for correctly diagnosing LB in children (10). We attribute this important difference to the specificity of the serologic assays used. Our laboratory has previously published papers on both the ELISA and Western blot assay used for this study (6, 8). These assays were developed and validated with disease control (non-Lyme disease) patients, stressing specificity as a primary objective. The results presented show that in the absence of documented EM, serology (of high specificity) should be used to rule out a diagnosis of LB for patients with chronic symptoms, thus preventing unnecessary, ineffective, and dangerous treatment with antibiotics for prolonged periods.

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## REFERENCES

1. **Abele, D. C., and K. H. Anders.** 1990. The many faces and phases of borreliosis. I. Lyme disease. J. Am. Acad. Dermatol. **23:**401–410.
2. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
3. **Canica, M. M., F. Nato, L. deMerle, J. C. Mazie, G. Baranton, and D. Postic.** 1993. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand. J. Infect. Dis. **25:**441–448.
4. **Cutler, S. J., and D. J. M. Wright.** 1994. Predictive value of serology in diagnosing Lyme borreliosis. J. Clin. Pathol. **47:**344–349.
5. **Dressler, H., R. Ackermann, and A. C. Steere.** 1994. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. J. Infect. Dis. **169:**313–318.
6. **Fawcett, P. T., K. M. Gibney, C. D. Rose, S. B. Dubbs, and R. A. Doughty.** 1992. Frequency and specificity of antibodies that cross-react with *Borrelia burgdorferi* antigens. J. Rheumatol. **19:**582–587.
7. **Fawcett, P. T., K. M. Gibney, C. D. Rose, J. D. Klein, and R. A. Doughty.** 1991. Adsorption with a soluble *E. coli* antigen fraction improves the specificity of ELISA tests for Lyme disease. J. Rheumatol. **18:**705–708.
8. **Fawcett, P. T., C. D. Rose, and K. M. Gibney.** 1995. Comparative evaluation of adsorption with *E. coli* on ELISA tests for Lyme borreliosis. J. Rheumatol. **22:**684–688.
9. **Gerber, M. A., and E. D. Shapiro.** 1992. Diagnosis of Lyme disease in children. J. Pediatr. **121:**157–162.
10. **Hsu, V. M., S. J. Patella, and L. H. Sigal.** 1993. "Chronic Lyme disease" as the incorrect diagnosis in patients with fibromyalgia. Arthritis Rheum. **36:** 1493–1500.
11. **Kantor, F. S.** 1994. Disarming Lyme disease. Sci. Am. **271**(3)**:**34–39.
12. **Laemmli, U. K., and M. Fayre.** 1973. Maturation of the head of bacteriophage T4. J. Mol. Biol. **80:**575–599.
13. **Marshall, E.** 1995. NIH gears up to test hotly disputed theory. Science **270:**228–229.
14. **Ostrov, B. E., and B. H. Arthreya.** 1991. Lyme disease: difficulties in diagnosis and management. Pediatr. Clin. North Am. **38:**535–538.
15. **Rose, C. D., P. T. Fawcett, K. M. Gibney, and R. A. Doughty.** 1994. The overdiagnosis of Lyme disease in children residing in an endemic area. Clin. Pediatr. **33:**663–668.
16. **Rose, C. D., P. T. Fawcett, K. M. Gibney, B. H. Singsen, S. B. Dubbs, and R. A. Doughty.** 1991. Use of Western blot and enzyme-linked immunosorbent assays to assist in the diagnosis of Lyme disease. Pediatrics **88:**465–470.
17. **Schoen, R. T., J. M. Aversa, D. W. Rahn, and A. C. Steere.** 1990. Treatment of refractory chronic Lyme arthritis with arthroscopic synovectomy. Arthritis Rheum. **33:**S85.
18. **Steere, A. C.** 1994. Lyme disease: a growing threat to urban populations. Proc. Natl. Acad. Sci. USA **91:**2378–2383.
19. **Steere, A. C., E. Dwyer, and R. J. Winchester.** 1990. Association of chronic Lyme arthritis with HLA-DR-2 alleles. N. Engl. J. Med. **323:**219–223.
20. **Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Shope, W. A. Andiman, M. R. Ross, and F. M. Steele.** 1977. Lyme arthritis: an endemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20:**7–17.
21. **Steere, A. C., E. Taylor, G. L. McHugh, and E. L. Logigian.** 1993. The overdiagnosis of Lyme disease. JAMA **269:**1812–1816.