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C_6 Test as an Indicator of Therapy Outcome for Patients with Localized or Disseminated Lyme Borreliosis

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Management of Lyme disease would benefit from a test to assess therapy outcome. Such a test could be employed to ascertain if treatment of early Lyme disease was successful and would be helpful to clinicians assessing patients with lingering posttreatment symptoms. We reported recently that levels of the antibody to C_6, a *Borrelia burgdorferi*-derived peptide that is used as an antigen in the C_6-Lyme diagnostic test, declined after successful antibiotic treatment of Lyme borreliosis patients. We assessed retrospectively the change in anti-C_6 antibody titers in 131 patients with either early localized disease (erythema migrans) or disseminated disease. All of these patients were treated with antibiotics and were free of the clinical signs shown at presentation within 12 weeks after the initiation of treatment. Decreases in reciprocal geometric mean titers (rGMT) of the anti-C_6 antibody were quantified for the subpopulation of 45 patients whose baseline rGMT were ≥80 and whose second serum specimens were obtained at least 6 months after the baseline specimen. Eighty percent of this patient group (36 of 45) experienced a ≥4-fold decrease in their rGMT ($P < 0.0003$). These results suggest that a change in the anti-C_6 antibody titer may serve as an indicator of therapy outcome for patients with localized or disseminated Lyme borreliosis.

Lyme borreliosis is a multisystem disease that is caused by the spirochete *Borrelia burgdorferi*. The disease is endemic in North America, Europe, and Asia. At present it is the most common vector-borne illness in the United States (5). For clinical purposes, the progression of Lyme borreliosis is divided into early localized, early disseminated, and late stages. The disease usually begins with a characteristic skin lesion, erythema migrans (EM). After several days or weeks, the spirochete may spread hematogenously, and patients may develop early disseminated disease with dermatologic, cardiac, neurologic, and rheumatologic involvement. Late disease can present chiefly as arthritis or neurologic symptoms (20).

Lyme disease is usually treated successfully with antimicrobial therapy, and the Infectious Diseases Society of America has recently issued practice guidelines for the therapy of Lyme disease (22). However, some treated individuals have persistent subjective complaints, and a few patients fail to respond to antibiotic therapy, as evidenced by signs of persistent infection (21, 22). For patients with late manifestations, the response to treatment is typically slower (22). It may take weeks or months, and it sometimes remains incomplete. Posttreatment Lyme disease syndrome, or chronic Lyme disease, is a condition that occurs in some patients after treatment for Lyme borreliosis. It is characterized by nonspecific symptoms of fatigue, sleep disorders, headache, memory and concentration difficulties, myalgia, and arthralgia. The cause of this syndrome is presently unknown, but antibiotic therapy does not seem to be helpful (10).

Management of Lyme disease would benefit from a test to assess therapy outcome. Such a test could be employed to ascertain whether treatment of early Lyme disease had been successful and thus prevented the transition to the late, more intractable form of the disease. It would also be helpful in assessing persistent, relapsing, or new symptoms after therapy. No such method is currently available.

Detection of the antibody to the C_6 peptide, which reproduces the IR_6 sequence (an immunodominant, conserved region of VlsE, the antigenic variation protein of *B. burgdorferi*), is currently used for serologic diagnosis of Lyme disease in humans (2, 12, 14; http://www.immunetics.com/c6/) and in canines (13). It has been reported recently that levels of the antibody to C_6 declined after successful antibiotic treatment of either Lyme borreliosis patients or animals that had been experimentally infected with *B. burgdorferi* (19). In humans, the anti-C_6 antibody titer diminished by a factor of ≥4 for successfully treated patients ($n = 30$) and by a factor of <4 for treatment-resistant patients ($n = 4$) (19). These preliminary results indicated that quantification of the anti-C_6 antibody titer as a function of time should be investigated as a test to assess the response to Lyme disease treatment (19).

We have now assessed retrospectively the change in anti-C_6 antibody titers in patients with early localized disease (EM) and with disseminated infections. C_6 titers were determined in serum samples that had been collected at the time of presentation and several weeks to months posttreatment. We assessed the decline in the titer as a function of clinical status posttreatment and as a function of the time elapsed between

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the collections of the first and second serum specimens. Here we report the results of this study.

MATERIALS AND METHODS

Patient population. The patient population consisted of 131 patients with either EM ($n = 39$) or disseminated disease ($n = 92$). Patients were assessed at the duPont Hospital for Children between 1991 and 2001. The median age was 8 years (range, 1.5 to 20). There were 44 female and 87 male patients. All patients had cases of Lyme disease that conformed to the clinical definition issued by the Centers for Disease Control and Prevention (CDC) (3). Serum specimens were obtained at the time of presentation and at a minimum of 1 month and a maximum of 18 months thereafter. Samples were obtained and tested in accordance with protocols approved by the Institutional Review Board of the duPont Hospital for Children. All patients received antibiotic therapy. All serum specimens were encoded such that C_6 antibody titers were obtained in a blinded fashion with respect to serum collection time or patient information.

Antibiotic treatment. The following treatment regimens were used: high-dose cefuroxime (13 patients; 30 mg/kg of body weight/day) orally for 20 days or low-dose cefuroxime (10 patients; 20 mg/kg/day), also for 20 days. The remainder (108 patients) received either oral amoxicillin at 50 to 60 mg/kg/day for 4 weeks, if they were 9 years old or younger, or oral doxycycline at 100 mg twice a day for 4 weeks, if they were older than 9 years. Treatment was considered successful if symptoms resolved within 12 weeks after the initiation of antibiotic treatment. This was assessed either through a follow-up visit or by phone. The assessment also took into account the possible appearance of new symptoms. Four patients continued to have evidence of synovitis for 6 months or longer.

Standard serologic tests. A standard Lyme enzyme-linked immunosorbent assay (ELISA) and Western blotting were performed as described previously (8, 9). Briefly, the ELISA was used to determine levels of immunoglobulin G (IgG) antibodies to *B. burgdorferi* in serum diluted 1:80 with phosphate-buffered saline (pH 7.4) containing a soluble fraction of *Escherichia coli*. Samples yielding an optical density (OD) greater than 0.20 OD unit were considered reactive, and results were reported as relative titers (9). Western blotting was performed to determine the presence of both IgG and IgM antibodies to electrophoretically separated components of a low-passage-number strain (B31; ATCC 35210) of *B. burgdorferi* blotted onto nitrocellulose membranes. Bound antibody was visualized by using a biotin-streptavidin detection system (8). Samples were considered Western blot positive if either the IgM or the IgG blot (or both) was positive.

Determination of anti-C_6 antibody levels and titers. Anti-C_6 antibody levels and titers were determined by using the C_6 ELISA from Immunetics, Inc. (Cambridge, Mass.). The U.S. Food and Drug Administration has approved this test for human use. Antibody levels were determined for the serum specimens collected at baseline. Titers were determined at each of two visits; the first visit was at diagnosis (baseline), and the second was 1 to 18 months later. All but two titer determinations were performed in duplicate. The reciprocal geometric mean titer (rGMT) of each duplicate determination is reported. Two patients had only one anti-C_6 antibody titer determination at baseline. In both cases the single determination was used in lieu of the geometric mean. Anti-C_6 antibody levels were determined according to the manufacturer's instructions. The result of the test is expressed as an index, which is calculated by dividing the OD of a given sample by that of a positive control included in the plate. The sample is considered positive if the index value is $\geq$1.10, negative if it is $\leq$0.90, and equivocal when it is between 0.91 and 1.09. Anti-C_6 antibody levels in human serum were determined, according to the manufacturer's instructions, at a serum dilution of 1:20. Therefore, in order to use the kit to determine antibody titers, the initial serum specimen dilution of 1:20 was subsequently serially diluted twofold with the buffer provided but supplemented with normal human serum at a dilution of 1:20. Thus, the serum concentration was maintained at 1:20 at all anti-C_6 antibody dilutions. Normal human serum (Sigma Chemical Co., St. Louis, Mo.) was tested with the C_6 kit to ensure that it did not contain anti-C_6 antibody. Apart from this modification, the manufacturer's instructions to determine anti-C_6 antibody levels at each serum dilution were followed verbatim. Serum titer was defined as the last serum dilution at which the C_6 test yielded a positive index. The lowest titer that could be determined was 1:20.

Data analysis. For any particular event, such as a fourfold decrease in the anti-C_6 antibody titer, statistical significance was defined as the probability of observing "m" or more events out of a possible "n" under the null hypothesis that the distribution of the number of events is binomial, with the probability of a single event equal to 0.5. Fisher's exact two-tailed test for 2×2 tables was used to test for association between a treatment group and a fourfold decrease in the anti-C_6 antibody titer. Statistical significance was defined as a P value of <0.05.

TABLE 1. Comparative sensitivities of the whole-cell Lyme ELISA, Western blotting, two-tiered algorithm, and C_6 test

Disease stage	No. of patients	No. (%) of patients positive by:			
		ELISA	Western blotting	Two-tiered algorithm	C_6 test
EM	39	9 (23)	11 (28)	4 (10)	17 (44)
Disseminated	92	80 (87)	90 (98)	78 (85)	81 (88)
Total	131	89 (68)	101 (77)	82 (63)	98 (75)

RESULTS

C_6 versus standard serology. Baseline assessment of patient serology yielded the following results. Of the 39 patients with EM, 9 (23%) were ELISA positive and 11 (28%) were positive by Western blotting. Only four of the ELISA-positive patients also were positive by Western blotting, i.e., satisfied the two-tiered diagnostic algorithm recommended by the CDC (Table 1). There were 17 (44%) EM patients who were C_6 positive at baseline. All but one of the 11 Western blot-positive patients were also C_6 positive. The mean (geometric) C_6 rGMT for all of the 39 EM patients at baseline was 40 (range, 10 to 8,611). C_6 rGMTs that were below 20 (negative) were arbitrarily set at 10 for this calculation.

Of the 92 patients with disseminated disease, 80 (87%) were ELISA positive, 90 (98%) were Western blot positive, and 78 (85%) were positive by the two-tiered algorithm (Table 1). Eighty-one patients (88%) were positive by the C_6 test. Overall, 82 patients (63%) were positive by the two-tiered algorithm, and 98 (75%) were positive by the C_6 test. The mean baseline rGMT for patients with disseminated disease was 380 (range, 10 to 7,421), which was about 9.5 times higher than that of the EM patients.

Decline in anti-C_6 antibody titer as a function of time between baseline and second sample collection. In order to ascertain whether the anti-C_6 antibody titer fell by a factor of 4 or more after antibiotic treatment, it was required that the baseline GMT be at least 1:80, because the lowest titer that could be determined was 1:20. Of the 131 specimens assessed, 91 satisfied that condition. Decreases in rGMT (rGMT1/rGMT2) as a function of time elapsing between the first and second serum collections are shown in Fig. 1.

In our initial study (19), it appeared that samples collected at least 21 weeks after the baseline collection showed $\geq$4-fold decreases in anti-C_6 antibody titers when patients were determined clinically to be cured. In this study, the median rGMT decrease gradually reached and surpassed fourfold in the 1- to 3-month-interval range and then oscillated around this level. After a 6-month interval, the median rGMT decrease remained above fourfold. Therefore, we considered 6 months to be the minimum interval after which to assess whether the decrease in the anti-C_6 rGMT correlated significantly with treatment outcome. There were 45 patients who satisfied the double requirement of having an anti-C_6 rGMT of $\geq$80 at baseline and a second serum sample that had been collected 6 months or longer thereafter.

Decrease in anti-C_6 rGMT as an indicator of treatment outcome for patients with EM or disseminated-infection symptoms. The distribution of patients with EM and disseminated

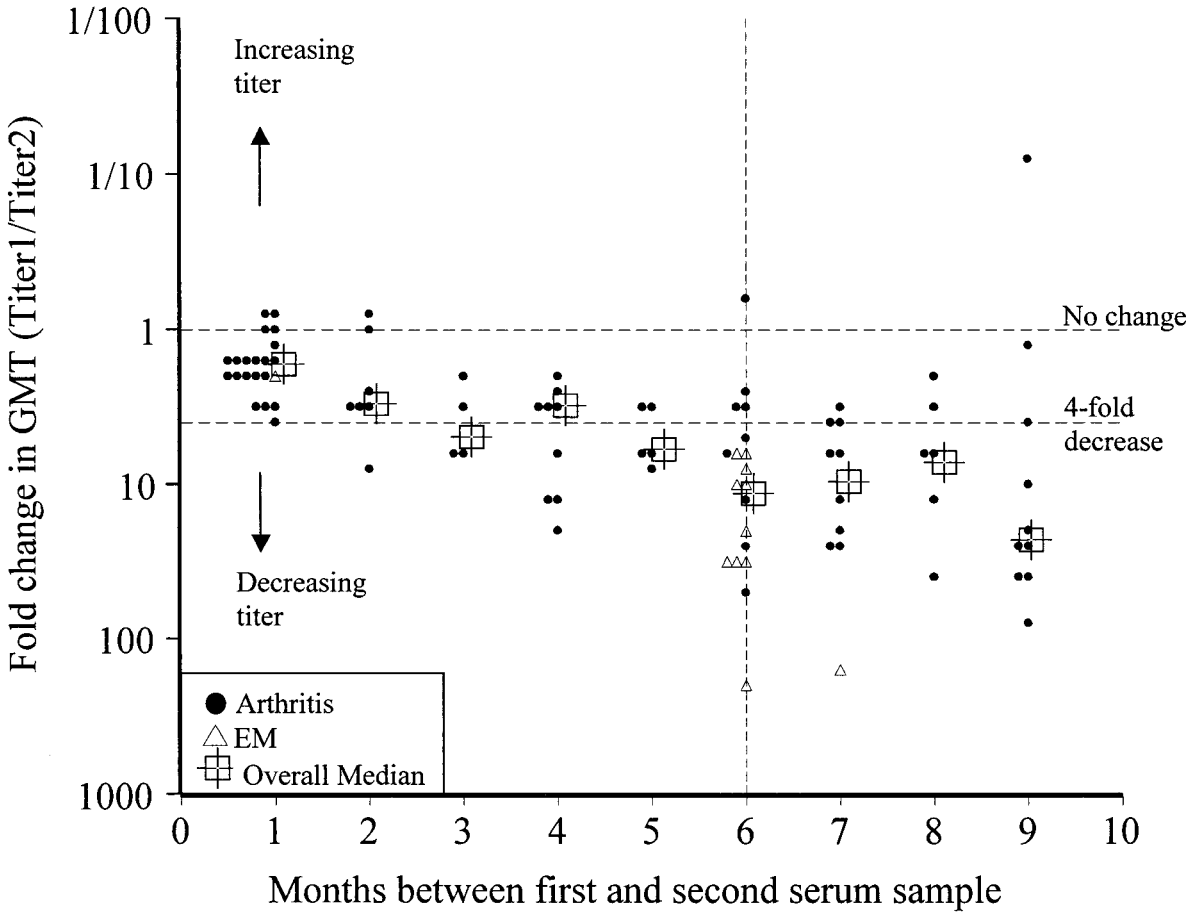

FIG. 1. Decrease in rGMT, expressed as rGMT1/rGMT2, as a function of the interval (in months) between the first and second serum sample collections.

disease who did or did not experience a ≥4-fold decrease in anti-C_6 rGMT at 6 months or longer after diagnosis is shown in Table 2. Of the 11 patients with EM, all experienced ≥4-fold decreases in their titers, as did 25 of the 34 patients with disseminated disease. Thus, of the 45 patients whose baseline rGMTs were ≥80 and whose second serum samples had been obtained at least 6 months later, 36 (80%) experienced ≥4-fold decreases in their anti-C_6 rGMTs. If the drop in rGMT were a random event, i.e., if the chance of a fourfold titer decrease were 50%, the probability of observing 36 or more patients satisfying this condition out of 45 is expressed by a *P* value of 0.00003. This is highly statistically significant. Patients with EM more consistently experienced ≥4-fold rGMT decreases (100%) than patients with disseminated disease (73.5%), but this difference did not reach a significant level ($P = 0.0867$ by Fisher's exact two-tailed test).

TABLE 2. Distribution of patients who did or did not experience a ≥4-fold decline in anti-C_6 antibody GMT at ≥6 months after diagnosis

Disease stage	C_6 GMT ≥4-fold lower than at baseline (no. of patients)		Total
	Yes	No	
EM	11	0	11
Disseminated	25	9	34
Total	36	9	45

DISCUSSION

The findings reported in this paper confirm and expand on observations made previously both by us and by others. A recent study by the CDC on the comparative diagnostic performance of a C_6 kinetic ELISA and two-tiered testing for Lyme borreliosis indicated that the C_6-based test performs as well as or better than the two-tiered algorithm (2). This diagnostic algorithm was introduced in 1995 at the recommendation of the CDC (4). The first tier is an ELISA based on *B. burgdorferi* whole-cell antigen extract. When this is positive or equivocal, it is followed by Western blotting, which is evaluated by using the banding criteria developed by Dressler et al. (6) and Engstrom et al. (7). In the present study, the C_6 test was more sensitive than the two-tiered test, especially for patients with EM (44 versus 10%). However, with the EM patient population, the fact that only an IgG standard ELISA was

performed may have diminished the sensitivity of the two-tiered test.

The C_6 test also performed well as a predictor of treatment outcome. Of the 11 EM patients whose baseline anti-C_6 rGMTs were ≥80 and whose second samples were taken ≥6 months after the baseline specimens had been collected (the "6/80 condition"), all (100%) experienced ≥4-fold decreases in C_6 titers. There were six additional EM patients who had positive C_6 tests at baseline. Two patients had the second sample taken only 1 month after baseline. In one of these cases, the anti-C_6 rGMT changed from 1,280 to 640 (a twofold decrease), and in the other the rGMT increased from 20 to 113. Clearly, the timing of sample collection with respect to the time of infection is important, and the result we observed in the latter case could relate to the possibility that the baseline serum specimen was obtained too soon after infection. In practical terms, it may be important to titrate not only a serum specimen at baseline but also one obtained early in the convalescent period (perhaps up to 1 month after collection of the first specimen), in case the baseline sample is obtained too soon after infection and might not exhibit an anti-C_6 titer high enough to permit quantification of the titer decrease 6 months after treatment initiation. The remaining four EM patients testing positive for C_6 all had serum sample pairs obtained 6 months apart, but their baseline rGMTs were below 80. The rGMT either diminished ($n = 3$) or stayed the same ($n = 1$). Thus, the overall trend was for the anti-C_6 titer to diminish, even when the 6/80 condition was not satisfied.

A smaller proportion (74%) of the patients with disseminated disease experienced ≥4-fold decreases in their rGMTs. This was not significantly different, as assessed by Fisher's exact two-tailed test, from the corresponding proportion of EM patients. However, it is evidence to suggest that the phenomenon of decrease in anti-C_6 antibody titers following treatment may be more apparent in patients who present in the earlier phases of Lyme borreliosis, provided the baseline serum sample is obtained at a time following infection at which the anti-C_6 antibody titer is at least 1:80.

In a recent study of posttreatment persistence of the anti-C_6 antibody response in Lyme disease patients with both early and late disease, Peltomaa et al. (18) reported results that at first glance appear squarely to contradict our findings. In a group of 15 patients with EM and 9 with facial paralysis, also an early sign of Lyme disease, the authors found that the anti-C_6 antibody reciprocal titer decreased <4-fold for 6 of 15 (40%) and 2 of 9 (22%) patients, respectively (8 of 24 [33%] for the group as a whole). If we analyze these results in terms of the null hypothesis we applied to our own data, the proportion of patients with early disease whose C_6 reciprocal titers fell by a factor of 4 or more, 66%, almost reaches statistical significance ($P = 0.076$). In point of fact, however, the proportion of such patients was greater than that reported. Three of the EM patients had negative anti-C_6 titers throughout the study. They appear to have been included among the patients whose titers did not decrease ≥4-fold. Such patients should not have been included in the study. In addition, the posttreatment specimen collected from one of the EM patients whose titer failed to decrease by a factor of 4 or more was taken before the fourth month after treatment. In our study also, the median fold change in rGMT for samples collected within such a short posttreatment period was less than fourfold. If, as we did in our study, such a patient is excluded from the analysis, the total number of EM patients is 11, and the number of patients whose titers fell fourfold or more is 9 (82%). The total proportion of early disease patients is therefore (9 + 7)/(11 + 9), or 80%. This is a significant fraction of the early patients ($P = 0.006$). Thus, for patients with early disease, the results of the study by Peltomaa et al. resemble ours.

Differences between the two studies are more ostensible for patients with late disease. Eighteen of a total of 21 (86%) had <4-fold decreases in anti-C_6 antibody titers. As before, several of these patients, among the group with treatment-responsive arthritis, had their posttreatment specimens collected around the fourth month after collection of the baseline sample. For all of these patients ($n = 8$), anti-C_6 antibody titers were described as decreasing <4-fold, but 6-month serum samples were not available.

Another issue that could have globally affected the results of this study is the manner in which the antibody titers were determined. From what can be gleaned from the description of methods, each serum specimen was not individually titrated by serial dilution. Rather, for each ELISA plate, a reference serum specimen was titrated and the titration curve was obtained. A single OD at a dilution of 1:200 was determined for each of the patient specimens, and the titers were obtained by linear extrapolation from that OD data point, presumably in parallel with the linear portion of the reference titration curve. Only those samples whose ODs were above that of the reference curve at a 1:200 dilution were serially diluted, and their titers were experimentally determined. To assess the extrapolation method, we used it to "calculate" the titers of all of our specimens that satisfied the 6/80 condition ($n = 45$), using as the point for extrapolation the OD at the dilution of 1:20 (this is the minimum dilution at which the OD is measurable in our system; the corresponding minimum dilution in the system used by Peltomaa et al. seems to have been 1:200). As a result, eight patients whose anti-C_6 rGMTs had decreased by a factor of ≥4 now showed <4-fold decreases, and one patient showed the inverse result. The titer calculation (extrapolation) thus yielded nine erroneous titer ratio values, a significant difference ($P < 0.02$) when assessed by using McNemar's test for correlated proportions (16). However, when the OD at a serum dilution of 1:40 was chosen as the point from which to extrapolate the titration curve and calculate the titer by extrapolation, only three titer ratios were erroneously calculated, an insignificant difference. Therefore, the method used by Peltomaa et al. may yield correct titer ratios if the extrapolation point is chosen appropriately. Unfortunately, the extrapolation point leading to the least number of titer calculation errors may not be known a priori.

On another issue, the authors point out that the "persistence of the anti-VlsE antibody response for months or years after antibiotic treatment cannot be equated with spirochetal persistence in Lyme disease" (18). We never equated the persistence of the anti-C_6 antibody with the persistence of infection. In our initial study (19), as well as in the study we report on here, we refer only to the magnitude of the anti-C_6 antibody titer change as having potential diagnostic value as a predictor of treatment outcome.

We had hypothesized (19) that the decline in anti-C_6 anti-

body titers in the wake of antibiotic treatment might be due to properties we attributed to VlsE, the antigenic variation protein of *B. burgdorferi* (23) from which C_6 is derived, and immunological memory of the antibody response to this antigen. We argued that VlsE should be constantly turned over by living spirochetes, so as to avoid accumulation of multiple VlsE variants on the spirochete surface (19). Surface accumulation of multiple VlsE variants would render antigenic variation inefficient. A possible consequence of VlsE turnover was that this antigen would not be available in large amounts at any time, relative to other antigenic components of the spirochete. Sparse availability of the VlsE antigen would make VlsE B-cell memory short-lived (19). This is so because the memory B-cell pool is maintained by stimulation with antigens stored as immune complexes on follicular dendritic cells (FDC). Upon spirochetal death, following antibiotic treatment, there would be a relatively scant reserve of VlsE antigen available to be stored in FDC and thus limited B-cell memory, leading to a quick decline in anti-C_6 antibody levels. Antibodies to other, more abundant *B. burgdorferi* antigens would be more persistent. Inevitably, the longer the infection remained untreated, the greater the opportunity to store VlsE in FDC, permitting as a consequence the generation of stronger VlsE (and C_6) B-cell memory. This hypothesis thus predicts, as both we and Peltomaa et al. (18) indeed observed, that patients with late disease should be less likely to show a posttreatment decline in anti-C_6 antibody titers than patients with early Lyme borreliosis.

Another interpretation we offered for the posttreatment decline in the anti-C_6 antibody response vis-à-vis the response to other *B. burgdorferi* antigens related to the possibility that the anti-C_6 response was either wholly or partially T-cell independent (19). Since T-cell-independent responses are poor elicitors of B-cell memory, this interpretation would also help to explain our observations. If a partly T-cell independent response were truly at play, it might help to explain why in our study the anti-C_6 antibody rGMT was as low as 380 for patients with disseminated disease (40 for EM patients), whereas titers appeared to be 10- to 60-fold higher for the patients with late disease in the study by Peltomaa et al. (18). T-independent antigens induce lower antibody responses in young children than they do in adults (1). The median age of the patients in the study of Peltomaa et al. was between 38 and 48 years (range, 3 to 62), as opposed to 9 years (range, 1.5 to 20) for the disseminated-disease patients of our study.

Of the 92 patients with disseminated disease in our study, 34 satisfied the 6/80 condition. As many as 79 had initial rGMTs of ≥80, and Fig. 1 clearly shows that the trend among these patients was to experience a gradual decrease in median rGMT as the time between baseline and second-sample collection increased. Among the patients with disseminated disease who satisfied the 6/80 condition, there were nine whose rGMTs did not decrease ≥4-fold. The information available on one of these patients indicates that the patient had persistent synovitis. An additional two also had lingering symptoms, but it was not clear whether these were derived from persistent infection or reinfection. For the remainder, there were no reports indicating failure to resolve the disease. Overall, 80% (36 of 45) of the patients who satisfied the 6/80 condition experienced ≥4-fold decreases in their anti-C_6 titers, with a P value of 0.00003. Therefore, this decrease in the anti-C_6 titer is highly correlated with response to therapy.

A limitation of our study, one that will be hard to overcome, is the lack of patients with proven failed therapy for Lyme disease. Patients with objective evidence of therapy failure (by culture or PCR) are extremely rare, and we have been unsuccessful thus far in acquiring serum specimens from such patients. One serum sample that we were able to obtain (15, 17) was already analyzed by us in a previous study (19).

The picture that we see emerging regarding the application of the C_6 test as a an indicator of therapy outcome is similar to the manner in which quantitative nontreponemal tests, e.g., the Venereal Disease Research Laboratory (VDRL) test or the rapid plasma reagin (RPR) test, are used for the assessment of syphilis treatment efficacy (11). To monitor syphilis treatment efficacy, quantitative nontreponemal tests are performed on serum specimens that are collected every 3 months for at least 1 year. After efficacious therapy for primary or secondary syphilis, a fourfold decline in the titer should be expected after the fourth month posttreatment, and a larger decline, up to eightfold, after the eighth month (11). For most early syphilis patients, the titers decline after treatment until little or no reaction is seen after the first year (11). In contrast, for latent or late syphilis patients, a more gradual decline in titers is observed (11), and titers may persist in approximately 50% of these patients after 2 years. This persistent seropositivity appears not to signify treatment failure and is likely to remain unchanged even after further treatment. Similarly, for patients with localized and perhaps also disseminated Lyme borreliosis, anti-C_6 antibody titers decrease fourfold within 6 months posttreatment in a significant majority, but not all, of the patients for whom antibiotic treatment for Lyme disease is efficacious. Conversely, a fourfold decrease in the anti-C_6 antibody titer within 6 months posttreatment may be interpreted as an indication of successful treatment, but a lesser decline is uninterpretable and may be due either to treatment failure or to persistence of the antibody for reasons other than persistence of infection. In patients with late Lyme disease, as per our results, and more dramatically in the patient population of Peltomaa et al., the decline in titers is more gradual.

With regard to the clinical application of the results of our study, two cautionary notes are pertinent. (i) Measurement of the anti-C_6 antibody titer as an indicator of response to therapy, as reported here, can be used only as part of a longitudinal assessment of the patient. A single titer is not informative of the patient's status after therapy. A great proportion of patients will have a persistently positive C_6 test, and this should not be equated with persistence of infection. (ii) As shown by us, the commercially available C_6 test can be used to determine anti-C_6 antibody titers. It should be borne in mind, however, that the "C_6 antibody index" per se, which is the direct readout of the C_6 test, is not a titer and may not be used as such. In conclusion, as shown by our results thus far, the C_6 test may serve as an indicator of therapy outcome in patients with localized or disseminated Lyme borreliosis, but further studies with larger patient populations are needed to clarify whether it is best suited for patients with early disease or whether it may also be used to predict the outcome of therapy of late Lyme borreliosis.

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REFERENCES

1. **Adderson, E. E.** 2001. Antibody repertoires in infants and adults: effects of T-independent and T-dependent immunizations. Springer Semin. Immunopathol. **23:**387–403.
2. **Bacon, R. M., B. J. Biggerstaff, M. E. Schriefer, R. D. Gilmore, M. T. Philipp, A. C. Steere, G. P. Wormser, A. R. Marques, and B. J. Johnson.** 2003. Serodiagnosis of Lyme disease by kiinetic ELISAs using recombinat VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with two-tiered testing using whole-cell lysates. J. Infect. Dis. **187:**1187–1199.
3. **Centers for Disease Control and Prevention.** 1997. Case definitions for infectious conditions under public health surveillance. Lyme disease. Morb. Mortal. Wkly. Rep. **46:**20–21.
4. **Centers for Disease Control and Prevention.** 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Morb. Mortal. Wkly. Rep. **44:**590–591.
5. **Centers for Disease Control and Prevention.** 2002. Lyme disease—United States, 2000. Morb. Mortal. Wkly. Rep. **51:**29–31.
6. **Dressler, F., J. A. Whalen, B. N. Reinhardt, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
7. **Engstrom, S. M., E. Shoop, and R. C. Johnson.** 1995. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J. Clin. Microbiol. **33:**419–427.
8. **Fawcett, P. T., K. M. Gibney, C. D. Rose, S. B. Dubbs, and R. A. Doughty.** 1992. Frequency and specificity of antibodies that crossreact with *Borrelia burgdorferi* antigens. J. Rheumatol. **19:**582–587.
9. **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.
10. **Klempner, M. S., L. T. Hu, J. Evans, C. H. Schmid, G. M. Johnson, R. P. Trevino, D. Norton, L. Levy, D. Wall, J. McCall, M. Kosinski, and A. Weinstein.** 2001. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N. Engl. J. Med. **345:**85–92.
11. **Larsen, S. A., B. M. Steiner, and A. H. Rudolph.** 1995. Laboratory diagnosis and interpretation of tests for syphilis. Clin. Microbiol. Rev. **8:**1–21.
12. **Liang, F. T., E. Aberer, M. Cinco, L. Gern, C. M. Hu, Y. N. Lobet, M. Ruscio, P. E. Voet, Jr., V. E. Weynants, and M. T. Philipp.** 2000. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi* SL. J. Infect. Dis. **182:**1455–1462.
13. **Liang, F. T., R. H. Jacobson, R. K. Straubinger, A. Grooters, and M. T. Philipp.** 2000. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **38:**4160–4166.
14. **Liang, F. T., A. C. Steere, A. R. Marques, B. J. Johnson, J. N. Miller, and M. T. Philipp.** 1999. Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* VlsE. J. Clin. Microbiol. **37:**3990–3996.
15. **Luft, B. J., R. J. Dattwyler, R. C. Johnson, S. W. Luger, E. M. Bosler, D. W. Rahn, E. J. Masters, E. Grunwaldt, and S. D. Gadgil.** 1996. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann. Intern. Med. **124:**785–791.
16. **McNemar, Q.** 1947. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika **12:**153–157.
17. **Melski, J. W., K. D. Reed, P. D. Mitchell, and G. D. Barth.** 1993. Primary and secondary erythema migrans in central Wisconsin. Arch. Dermatol. **129:**709–716.
18. **Peltomaa, M., G. McHugh, and A. C. Steere.** 2003. Persistence of the antibody response to the VlsE sixth invariant region (IR_6) peptide of *Borrelia burgdorferi* after successful antibiotic treatment of Lyme disease. J. Infect. Dis. **187:**1178–1186.
19. **Philipp, M. T., L. C. Bowers, P. T. Fawcett, M. B. Jacobs, F. T. Liang, A. R. Marques, P. D. Mitchell, J. E. Purcell, M. S. Ratterree, and R. K. Straubinger.** 2001. Antibody response to IR_6, a conserved immunodominant region of the VlsE lipoprotein, wanes rapidly after antibiotic treatment of *Borrelia burgdorferi* infection in experimental animals and in humans. J. Infect. Dis. **184:**870–878.
20. **Steere, A. C.** 2001. Lyme disease. N. Engl. J. Med. **345:**115–125.
21. **Steere, A. C., R. E. Levin, P. J. Molloy, R. A. Kalish, J. H. Abraham III, N. Y. Liu, and C. H. Schmid.** 1994. Treatment of Lyme arthritis. Arthritis Rheum. **37:**878–888.
22. **Wormser, G. P., R. B. Nadelman, R. J. Dattwyler, D. T. Dennis, E. D. Shapiro, A. C. Steere, T. J. Rush, D. W. Rahn, P. K. Coyle, D. H. Persing, D. Fish, B. J. Luft, et al.** 2000. Guidelines from the Infectious Diseases Society of America. Practice guidelines for the treatment of Lyme disease. Clin. Infect. Dis. **31**(Suppl. 1)**:**1–14.
23. **Zhang, J. R., J. M. Hardham, A. G. Barbour, and S. J. Norris.** 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. Cell **89:**275–285.