

SEROLOGICAL FOLLOW-UP OF PATIENTS WITH ERYTHEMA MIGRANS: PERSISTENCE OF ANTIBODY RESPONSE TO *BORRELIA BURGDORFERI* IN LYME DISEASE PATIENTS

I. CHRISTOVA and R. KOMITOVA¹

Microbiology Department, National Center of Infectious and Parasitic Diseases, Sofia, and ¹Department of Infectious Diseases, Medical University, Sofia, Bulgaria

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The rate of seroconversion before treatment and antibody kinetics after treatment were analyzed and possible interpretations of serologic findings was proposed. Serum samples from 219 patients with *Erythema migrans* were tested by ELISA for antibodies against *B. burgdorferi*. Twenty-eight (28%) to 55% of the patients showed isolated IgM antibody response, 3-5% showed isolated IgG response, 6-16% showed concomitant IgM and IgG responses, and 24-63% tested seronegative depending on number of days passed after the onset of Lyme borreliosis. One year after treatment, 38% of the patients still had IgG response and 10% had IgM antibodies against *B. burgdorferi*. Furthermore, 4 of 106 seronegative patients revealed IgM response three months after treatment despite lack of signs or symptoms of active Lyme borreliosis. We concluded that persistence of antibody response is not indicative of treatment failure, although regular clinical and laboratory examinations, including PCR, should follow successful treatment.

Lyme borreliosis is a multi-system tick-borne disease caused by a variety of *Borrelia* species, assigned as *Borrelia burgdorferi* sensu lato. The bacterial species, transmitted by the tick, *Erythema migrans* (EM), causes symptoms such as a red rash or lesion at the site of a tick bite, which usually appear within 3-30 days. The skin lesion represents early inflammatory reaction due to persistence and multiplication of the etiological agent. EM is the clinical hallmark of early Lyme borreliosis.

Serological diagnosis of *Lyme borreliosis* is complicated by a number of limitations. First, antibody response to *B. burgdorferi* is slow and appears late in the course of the disease, leading to low diagnostic sensitivity. A significant number of patients with early Lyme borreliosis do not have antibodies to *B. burgdorferi*. Early antibiotic therapy may have dampening effects on antibody response. Consequently, a negative serological test does not exclude diagnosis of early *Lyme borreliosis*. Additionally, antibody persistence

makes it difficult to distinguish between active and past infection. Antibodies may persist in untreated (1) and in successfully treated EM patients. In some patients IgG and even IgM antibodies may persist years after treatment (2-5).

Kinetics of specific antibodies after treatment of patients with *Lyme borreliosis* is valuable in determination of either good response to therapy, reinfection, or treatment failure. It is important to distinguish persistent infection from post infectious sequelae. It is also important to realize the extent to which serology could serve as a helpful tool. In order to elucidate this further, we conducted a one-year follow-up study of patients with physician-diagnosed EM that were treated with suggested doses and regimens of antibiotics.

MATERIALS AND METHODS

Patients

A total of 219 patients with EM were included in the study. Patients were evaluated at the Department of

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Infectious Diseases, Medical University, Sofia and at the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria. All patients satisfied the definition criteria for Lyme disease. All patients were treated with antibiotics in accordance with recommended guidelines. All patients showed regression of EM lesion within a few weeks after the treatment.

Serum samples

Serum samples were collected from patients prior to treatment and then at 3 months, 6 months, and 12 months following treatment. Patients were evaluated for signs of *Lyme borreliosis* at each visit.

Serologic testing

Sera tested for IgM and IgG antibodies to *B. burgdorferi* by ELISA based on ultrasonicated Bulgarian *B. garinii* isolated BT6 as described before (6). Hundred µl of each 1:200 diluted serum specimen, positive and negative controls, was added to the antigen-coated microtitre plate. The plates were incubated at room temperature for 1h and subsequently washed three times with phosphate-buffered saline containing Tween 20. Next, 100 µl of rabbit anti-human IgM or IgG horseradish peroxides conjugate (DAKO, Denmark) was added to each well, and the plates were then incubated for 30 min. After a final wash, 100 µl of OPD

substrate (0,4 mg/ml o-phenyldiamine and 0,1 µl/ml hydrogen peroxide) was added and color was allowed to develop for 10 min. To stop the reaction, 50 µl of 2 N sulfuric acid was added and the plates were read with a spectrophotometer at 492 nm. The cutoff optical density readings were 5 SD (for IgM) and 3 SD (for IgG) above the mean optical density of samples from 8 healthy control subjects included on the same plate.

RESULTS

One hundred-four (47%) of the 219 patients with EM were positive for IgM and 26 (12%) were positive for IgG prior to treatment. In patients (n=87) with up to 10 days history of symptoms, 28% showed an isolated IgM response, 3% an isolated IgG response, 6% showed concomitant IgM and IgG response, and 63% were seronegative (Tab. I). In patients with 10-20 days duration of the disease the proportion of IgM positive increased to 39%, and the relative amount of both IgM and IgG positive remained unchanged.

Prevalence of IgM positive over seronegative findings appeared in patients 20-40 days after the onset of the disease when 51% of the patients were IgM positive and 37% were negative. Fifty-five percent (55%) of the patients tested more than 40

ELISA results	Days after onset of the disease				Average No. (%)
	0-10 No. (%)	10-20 No. (%)	20-40 No. (%)	40-60 No. (%)	
Only IgM positive	24 (28%)	20 (39%)	22 (51%)	21 (55%)	87 (40%)
Only IgG positive	3 (3%)	2 (4%)	2 (5%)	2 (5%)	9 (4%)
Both IgM and IgG positive	5 (6%)	3 (6%)	3 (7%)	6 (16%)	17 (8%)
Seronegative	55 (63%)	26 (51%)	16 (37%)	9 (24%)	106 (48%)
TOTAL	87 (40%)	51 (23%)	43 (20%)	38 (17%)	219 (100%)

Tab. I. *B. burgdorferi* IgM and IgG ELISA at the time of diagnosis (prior to treatment) of 219 patients with EM.

days after the onset of the disease showed an isolated IgM response. Only 24 % of the patients were seronegative and the proportion of IgM-IgG positive patients started to increase to as high as 16%.

All of the patients received appropriate antibiotic treatment and were repeatedly tested for up to 12 months after the treatment in order to detect persistence of antibodies (Fig.1). Serological testing was conducted 3 months after the treatment. Fifty-seven (55%) were IgM positive and 21 (81%) of the IgG positive. Also, 4 (4%) of originally IgM negative patients converted to positive despite the lack of clinical symptoms.

Control testing was conducted 6 months after the treatment. Twenty-nine (28%) of initially IgM positive patients and 16 (62%) of initially IgG positive remained positive.

This was repeated 12 months after the treatment. Ten (10%) of the patients that had shown IgM response were still IgM positive and 10 (38%) of the patients that had shown IgG response were still IgG positive. In addition, 3 (1, 4%) of the examined patients with early *Lyme borreliosis* were an exception from the described dynamic of antibody response. After antibiotic treatment 3 patients reverted to seronegative. Two patients between the 4th and 8th month and a third patient

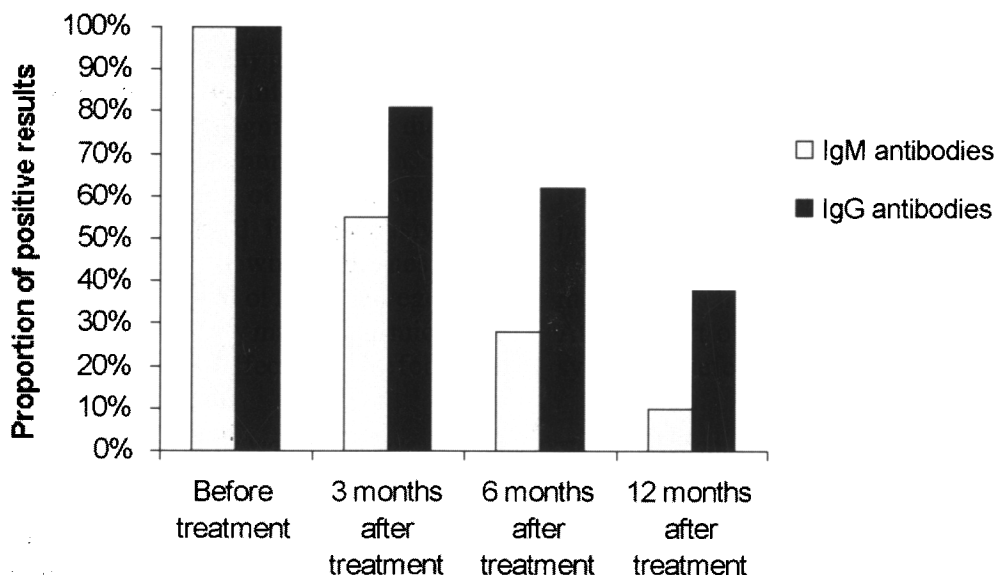
between the 14th and 18th month after the onset of the disease, showed clinical manifestations that are typically shown early in Lyme disease, which include meningitis, peripheral neuropathy, or neuritis. After antibiotic treatment they reverted to seronegative, but again once (two patients) and twice (one patient) - between the 4th and 8th month and between 14th and 18th month after the onset of the disease, presented with clinical manifestations – early disseminated Lyme disease – which were mentioned above. At this level, specific antibodies against *B. burgdorferi*, which were detected by ELISA, disappeared after antibiotic treatment.

DISCUSSION

In the present study we evaluated the rate of seroconversion in patients with EM before treatment and antibody kinetics after treatment. Although diagnosis of patients with EM is dependent on recognition of the skin lesion, such studies are necessary to understand pathogenesis of infection, analyze serologic findings that could be expected at various intervals before and after treatment, and elucidate possible interpretations of the results.

The study showed that about half (47%) of the patients with early *Lyme borreliosis* (EM) had IgM antibodies to *B. burgdorferi* and a low

Fig. 1. Kinetics of IgM and IgG antibody response of patients with EM during 1-year follow-up.



proportion of them (12%) had IgG antibodies. The rate of seronegative findings detected in the study is comparable to those in studies of European authors (4,7,8) and somewhat higher than those in earlier American studies (9-11). Since up to half of the patients may not have serum antibodies to *B. burgdorferi*, treatment must be initiated on the basis of clinical findings. About two-thirds of the patients with up to 10 days duration of the disease were seronegative. The rate of seronegatives decreased as the length of duration after the onset of the disease increased. Approximately every second, third and fourth patient tested negative 10-20 days, 20-40 days, and 40-60 days, respectively, after the onset. This could be based on the delay of antibody response in Lyme borreliosis. While IgM antibody response began shortly after infection, levels of antibodies were below diagnostic sensitivity of serologic test. The IgM antibodies gradually became elevated, reaching detectable levels in most patients at day 20 of the infection. Concomitant IgM and IgG antibody responses were detected in 6-7% of the patients with up to 40 days duration of the disease. This rate suddenly increased in patients with a longer duration of the disease, demonstrating the initiation of specific- IgG antibody response later in the course of the disease.

The isolated IgG response detected in 3-5% of the patients with early *Lyme borreliosis* may be a consequence of a previous asymptomatic *B. burgdorferi* infection or could be due to cross-reactivity to other spirochetal antigens. The fact that the rate of isolated IgG seropositivity remained almost unchanged, shows that most probably it represents serologic background, i.e. antibody level of Bulgarian patients. Bulgaria is endemic for Lyme borreliosis and rate of *B. burgdorferi* infection in tick vectors is about 40% (12).

All patients but three recovered after treatment with no discernable signs or symptoms of active or persistent *Lyme borreliosis* throughout the follow-up. Nevertheless, specific antibodies slowly declined and one year after the treatment, IgM antibody response could be detected in 10% of the IgM-positive patients prior to treatment and in 38% of the IgG-positive. Other studies have shown persistence of specific antibodies in a wide range – from 16% to 59% (9, 10, 14, 15) of patients have been detected seropositive one year after successful

treatment. Surprisingly, Kalish et al. (5) reported persistence of IgG and even IgM antibody responses, 10-20 years after treatment of early *Lyme borreliosis*. Long-lasting IgG antibody response after infectious diseases is typical, but persistent IgM response is unusual. In such cases, there have been no clinical signs of active infection or reinfection. Analysis of the antibody response by immunoblot has shown the same pattern of band reactivity as the original antibody response at the early onset of the disease (5). A possible explanation of the long-lasting IgM antibody response in *Lyme borreliosis* is the persistence of IgM-expressing memory cells (16,17). Specific IgM and IgG responses many years after successful treatment may result from memory T and B cells.

Interestingly, 4 of 106 seronegative EM patients showed IgM response three months after treatment even when no signs of infection could be detected. Development of IgM antibody response in these patients could mean that antibiotic treatment was sufficient to suppress the etiological agent and progression of the disease but could not stop production of the specific antibodies. It is well known that antibiotic treatment has dampening effects on the immune response. Furthermore, three of the originally seropositive patients that were treated and consequently became seronegative at the follow-up examination later revealed clinical symptoms of disseminated *Lyme borreliosis* with seropositivity once (2 patients) or twice (1 patient). This could be due to reinfection by another unnoticed tick bite. Another explanation is the possible negligence of the required daily regimen of antibiotics, as patients were treated on an outpatient basis. We believe that the seropositivity is a consequence of administering the antibiotics late in the course of the disease (days 40-60). Therefore, we recommend repeated serological follow-up examinations even in seronegative patients with early *Lyme borreliosis*.

In conclusion, IgM was the dominant antibody response in patients with EM even though more than half of the patients were seronegative when tested up to 20 days after onset of disease, with a small proportion showing IgG antibody response. By the time of convalescence, one year after treatment, a significant part of the originally seropositive patients still showed antibody response, mostly IgG but also IgM. Furthermore, a few

seronegative patients developed IgM antibodies to *B. burgdorferi* despite the lack of clinical symptoms of *Lyme borreliosis*. We conclude that antibody response could persist in some *Lyme borreliosis* patients despite antibiotic treatment and lack of clinical symptoms. Therefore, repeated antibiotic treatment based solely on positive serology should be avoided. At this level, PCR should be performed. Antibiotic treatment should be considered only if PCR testing in blood or urine is positive for *B. burgdorferi*. Diagnosis of *Lyme borreliosis* is primarily clinical. To prevent progression of the disease despite prompt therapy, regular clinical and laboratory examinations, including PCR, after treatment are necessary.

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