

## Changes in *Borrelia burgdorferi*-specific Serum IgG Antibody Levels in Patients Treated for Acrodermatitis Chronica Atrophicans

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The kinetics of *Borrelia burgdorferi*-specific serum IgG antibody values in 74 patients treated for acrodermatitis chronica atrophicans was analysed by means of enzyme-linked immunosorbent assay. At the last clinical control, there had been no clinical signs of active infection. The serological follow-up time ranged from 12 months to 5 1/2 years (median 2 years and 1 month). In 68 (92%) of the 74 patients, a significant decrease of the specific antibody values was found within 3 years after the initiation of therapy. In 53 (72%) of the patients, this decrease was found within 15 months. Most of the patients remained seropositive during the follow-up period. The results show that a significant decline of the levels of serum IgG antibodies to *Borrelia burgdorferi* can be expected in the majority of patients who do not exhibit clinical evidence of persistent infection after antibiotic treatment of acrodermatitis chronica atrophicans.

**Key words:** Lyme; borreliosis.

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Acrodermatitis chronica atrophicans (ACA) is a late and chronic cutaneous manifestation of Lyme borreliosis (1), and in Europe it is the most common late manifestation of the infection. It is usually located to the acral parts of the limbs. The typical clinical picture consists of inflammatory skin changes with a bluish-red erythema, often accompanied by swelling, and atrophy of the skin in long-standing disease. Concomitant extracutaneous manifestations are frequent; at least half of the patients with ACA have a usually mild neuropathy, and joint manifestations may also occur. At diagnosis, the skin lesions have often been present for years and sometimes decades. Spirochetes have been cultured from an ACA lesion of more than 10 years' duration, and spirochetes can thus survive in the skin for a very long period of time (2).

Elevated values of serum IgG antibodies to *Borrelia burgdorferi* have been established so far in practically all patients with ACA, and the values are often remarkably high (3–8). IgM reactivity is found in some sera but has been shown in most cases to be caused by IgM rheumatoid factor (6, 9). In serological follow-up after treatment of ACA, a long persistent specific antibody production has been found, although a decline of the antibody values has usually been noted (4, 5, 7). It is known from other manifestations of Lyme borreliosis as well that seropositivity can persist for a long time after antibiotic treatment (10–13). However, a more detailed analysis of the effects of treatment on the antibody response against *B. burgdorferi* in a large number of patients with ACA has not been reported, and the aim of the present study was to fill this gap. The assumption

behind the study was that knowledge of the post-treatment antibody kinetics in ACA might be valuable in the follow-up of ACA patients after antibiotic treatment.

### MATERIALS AND METHODS

#### Patients

Seventy-four patients treated for ACA during the years 1983 to 1990 were included in the study. All patients had been diagnosed, treated and followed-up after therapy by the authors at the Department of Dermatology at Södersjukhuset. The selection of these 74 patients was based on the availability of frozen and stored sera drawn before antibiotic treatment and on several occasions during a period of at least one year after treatment. The ACA diagnosis was based on the clinical and histopathological pictures and on elevated values for IgG antibodies to *B. burgdorferi* in serum determined by ELISA. The median age of the patients was 63 years, ranging from 24 to 80 years. There were 52 women and 22 men. The median duration of the ACA lesions before treatment was 2 years, ranging from half a year to >20 years.

**Antibiotic therapy.** All patients had received a 3–4 week course of antibiotics. Fifty-six patients had received initial oral treatment: phenoxymethyl-penicillin or doxycycline. Eighteen patients had been given initial intravenous therapy for 2 weeks (benzylpenicillin, cefotaxime or cefuroxime) followed by oral therapy (doxycycline or phenoxymethyl-penicillin). Eighteen patients had received repeated courses of antibiotic treatment; 15 of these were given initial oral therapy. One of these patients had received penicillin orally because of erysipelas while waiting for a planned intravenous treatment of the ACA, which was later carried out as intended. Another patient, primarily treated with phenoxymethyl-penicillin, had later received lymecycline and doxycycline due to persistent swelling of the heel. In 2 patients, treated in 1984 with phenoxymethyl-penicillin, this treatment had been repeated within 4 months because of persistent skin lesions. The remaining additional courses of antibiotic therapy, beta-lactam antibiotics intravenously and/or doxycycline orally, had been given due to signs or symptoms from joints or the nervous system.

**Follow-up.** After treatment the patients had been followed clinically and serologically. The last clinical control was performed 12 months to 5 years and 7 months after initiation of antibiotic treatment (median 2 years and 4 months). At this last clinical control, the assessment for all patients had been that there were no clinical indications of persistent infection.

#### Serum specimens

One serum sample drawn before treatment and 3–5 post-treatment serum samples from each patient were analysed. The post-treatment samples had been drawn at the following points of time after initiation of antibiotic treatment: 1–3 months (70 patients); 4–8 months (67 patients); 9–15 months (72 patients); 1.5–3 years (64 patients); 4–5 years (10 patients). The median value for the period after initiation of treatment when the last serum sample had been drawn was 2 years and 1 month, ranging from 12 months to 5 years and 7 months. The serum samples had been frozen and stored at  $-70^{\circ}\text{C}$ .



Table I. No. (%) of patients treated for acrodermatitis chronica atrophicans with a significant decrease of serum IgG antibodies to *Borrelia burgdorferi* at different time intervals after therapy

Antibody levels were measured by ELISA and given as absorbance values

	No.	Time after therapy			
		1-3 months	4-8 months <sup>a)</sup>	9-15 months <sup>b)</sup>	1.5-3 years <sup>c)</sup>
All patients	74	0	19 (26%)	53 (72%)	68 (92%)
Disease duration					
<2 years	34	0	8 (24%)	23 (68%)	31 (91%)
≥2 years	40	0	11 (28%)	30 (75%)	37 (93%)
Initial abs. values					
<0.850	37	0	11 (30%)	29 (78%)	33 (89%)
≥0.850	37	0	8 (22%)	24 (65%)	35 (95%)

<sup>a)</sup> Seven patients were not investigated after 4-8 months, of whom 2 had a significant decrease after 9-15 months.

<sup>b)</sup> Two patients were not investigated after 9-15 months, of whom one had a significant decrease after 1.5-3 years.

<sup>c)</sup> One of 6 patients in whom a significant decrease was not established was followed serologically <1.5 years.

#### Enzyme-linked immunosorbent assay

The sera had previously been examined for antibodies to *B. burgdorferi* during the clinical care of the patients. In the present study, all sera were re-analysed for specific IgG antibodies to *B. burgdorferi* by the use of ELISA under standardized conditions. Sera drawn on different occasions from one and the same patient were analysed simultaneously and all sera in continuity. An indirect ELISA with sonicated whole cell borreliae, strain ACA I, as antigen was used. Antigen preparation and

ELISA were performed as previously described (14), except for a higher dilution of the sera in the present study, to make possible a quantitative differentiation of the high antibody levels. In brief, microtitre plates were coated with antigen overnight. Sera diluted 1/5000 were added and the plates were incubated at 20°C for 1 h. After additional incubations with alkaline phosphatase-conjugated swine anti-human IgG (Orion, Helsinki, Finland) and substrate, the absorbance value at 405 nm was read (Titertek Multiscan, Flow Laboratories, UK). Antibody levels are given as absorbance values in the text.

In a previous study with sera diluted 1/1000, a cut-off level was defined as the 98th percentile of the absorbance values in 100 controls (14). In the higher dilution of the sera in the present study, that previously defined cut-off level corresponded to an absorbance value of about 0.060. However, at this low antibody level, the determined antibody values may be less exact in the higher dilution of the sera. Thus, the value 0.060 is regarded as an approximate - not exact - upper limit of normal values (cut-off). A ≥ twofold change of absorbance values was regarded as significant.

#### Statistical analysis

The correlation between pretreatment antibody levels and disease duration was analysed by linear regression. The chi-squared exact test was used for the comparisons between groups of patients according to disease duration or pretreatment antibody levels as regards the decrease of the antibody levels (in Table I). The antibody levels in the last serum

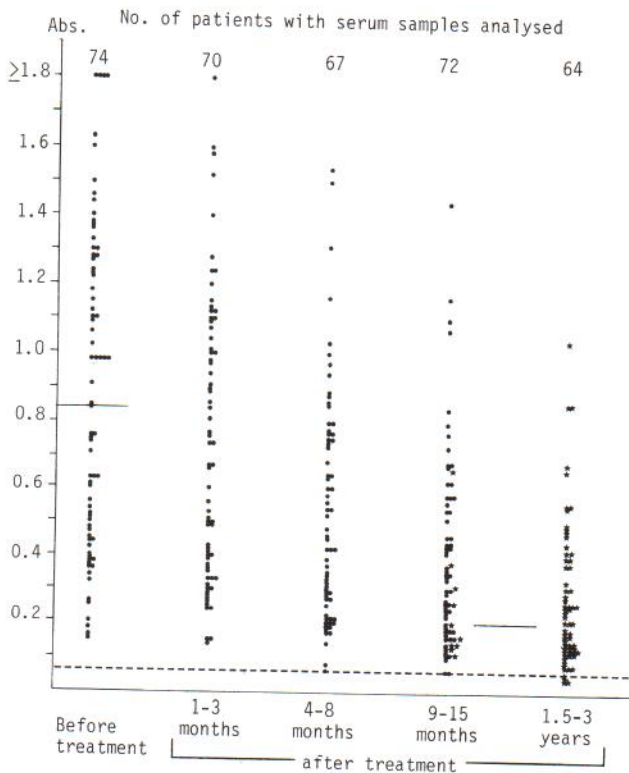


Fig. 1. Serum IgG antibodies to *Borrelia burgdorferi* in 74 patients with acrodermatitis chronica atrophicans before therapy and at follow-up up to 3 years after therapy. Antibody values were measured by ELISA and given as absorbance values. \* indicates value of last serum sample of each of the 74 patients during this follow-up period. The horizontal bars mark the median values of the first and last serum samples of all patients. The dotted line marks the approximate upper limit for normal values.

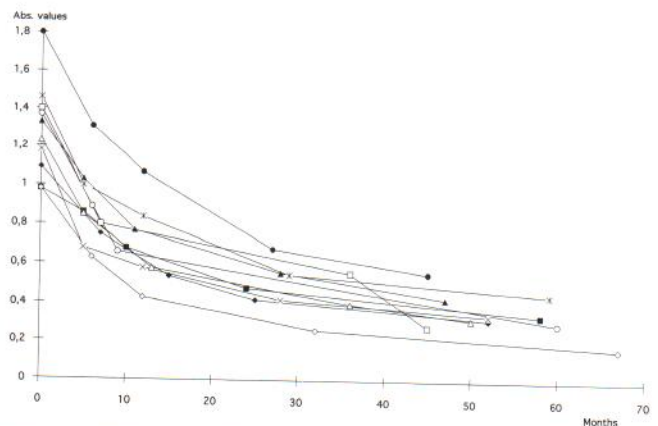


Fig. 2. Kinetics of the *Borrelia burgdorferi*-specific serum IgG antibody values in 10 patients with acrodermatitis chronica atrophicans followed for more than 3.5 years after antibiotic treatment. Antibody values were measured by ELISA and given as absorbance values.



samples were compared for patients with pretreatment values above and below the median level by a two-tailed independent *t*-test.

## RESULTS

The levels of IgG antibodies to *B. burgdorferi* in all serum samples drawn until 3 years after treatment are given as absorbance values in Fig. 1.

### *Specific antibody values before therapy*

The median absorbance value in the 74 serum samples drawn before treatment was 0.845 (range 0.150 to >1.800). There was no statistically significant correlation between disease duration and pretreatment antibody values ( $p=0.162$ ).

### *Specific antibody values after therapy*

After treatment, the specific antibody levels in the group of patients declined, as illustrated in Fig. 1. Not in any of the post-treatment serum samples from any patient was a significant increase of the antibody levels found. A significant decrease was seen in sera from 68 out of the 74 patients (92%). The time required after treatment for the significant decrease to take place is given in Table I. Thus in sera from 53 patients (72%), the specific antibody values had decreased significantly within 15 months after therapy. Of the remaining 21 patients, 20 had serum samples drawn 1.5–3 years after treatment, and, by that time, 15 of these 20 patients had also developed a significant decline of the specific antibody values.

Despite decreasing values of specific serum antibodies in the majority of the patients, the last serum sample analysed in most cases still revealed values above the approximate cut-off level (0.060). In the sera from 10 patients followed serologically for 4–5 years, all with high initial specific antibody values, a continuous decrease of the antibody levels was found, but none reached seronegativity (Fig. 2).

### *Specific antibody values after therapy compared to duration of the skin manifestations and to initial antibody values*

In Table I patients with a disease duration <2 years and patients with a disease duration  $\geq 2$  years are compared with regard to the number of patients with a significant decrease of the absorbance values at different points in time after treatment. The same comparison is made for patients with pretreatment absorbance values <0.850 versus patients with pretreatment absorbance values  $\geq 0.850$ . None of the comparisons revealed statistically significant differences. Of the 6 patients in whom a significant decrease was not established, 3 had a duration of the skin lesions  $\geq 2$  years and 2 pretreatment antibody levels  $\geq 0.850$ .

The specific antibody values in the last serum samples analysed were higher in the group of patients with pretreatment values >0.850 than in the patients with pretreatment values <0.850. The difference was statistically significant ( $p < 0.001$ ).

### *Patients in whom a significant decline of specific serum antibody values was not established*

In sera from 6 patients, a significant decline of the specific antibody levels was not established. In one of these patients, a serum sample drawn after 3 years showed a decrease of the absorbance value to 1.040 from a pre-treatment value of 1.860. A later serological control of this patient, after the completion of this study, showed a further slow decline of the antibody level. Two patients had the last serum samples drawn 2.5 years after therapy. Their initial specific antibody values were 0.910 and 0.440, respectively, and the last post-treatment values 0.660 and 0.380, respectively. In the remaining 3 patients, whose pre-treatment serum samples showed absorbance values between 0.150 and 0.360, the last serum samples were drawn less than 2 years after treatment. In these 3 patients as well, the absorbance values in the last serum samples were stationary or only insignificantly decreased.

Three of these 6 patients had been treated with oral doxycycline 200 mg daily for 3 weeks, 2 patients with intravenous cefuroxime 9 g daily for 2 weeks followed by oral doxycycline 200 mg daily for another 2 weeks and one patient with intravenous benzylpenicillin 12 g daily for 2 weeks followed by oral phenoxymethyl-penicillin 3 g daily for another 2 weeks.

### *Patients treated with two or more courses of antibiotics*

Eighteen of the 74 patients had received repeated courses of antibiotics. Of these 18 patients, 14 (78%) had pretreatment antibody values  $\geq 0.850$  compared to 23 of the 56 patients (41%) who were given only one course of antibiotics. A significant decrease of the specific serum antibody values 9–15 months after the initial treatment was established in 13 of the 18 patients (72%) who had received repeated treatment and in all 18 (100%) after 1.5–3 years. In 10 patients, additional antibiotic treatment had been given at a time when a significant decline of the specific antibody values had already occurred: in one case due to a persistent swelling of the heel and in the remaining 9 cases due to the fact that extracutaneous signs or symptoms, such as polyneuropathy or joint manifestations, had given rise to uncertainty about the sufficiency of the treatment.

## DISCUSSION

It is still not finally settled what comprises the optimal antibiotic treatment of the various manifestations of Lyme borreliosis (15). In evaluating the therapeutical results, the main point is certainly the clinical signs and symptoms, but differentiation of residual infection from post-infectious manifestations or sequelae and from non-borrelial illness can sometimes be difficult. It would therefore be of value if, as in the follow-up after treatment of syphilis, serology could serve as a complement to the clinical judgement.

In the present study, we have analysed the reactions of the specific serum IgG antibody levels after antibiotic treatment in 74 patients with ACA. In 68 (92%) of the patients, a significant decrease of the specific antibody values could be established within 3 years after initiation of antibiotic treatment. In 53



(72%) of the 74 patients, the significant decrease had occurred within 15 months after treatment.

A significant decrease of the specific antibody values seemed to occur without marked influence from either disease duration or pre-treatment values. As might be expected, however, the group of patients with the higher initial specific antibody values also had higher persistent values in the last serum sample analysed. The majority of the patients did not reach seronegativity during the follow-up period. It has previously been pointed out that positive values for antibodies to *B. burgdorferi* do not necessarily mean actual infection and may often falsely be thought to explain a presently existing disease (16). Thus, this is of special importance to remember in a patient previously treated for ACA. Ten patients in this study were followed serologically for 4–5 years and were still seropositive. Although a continuous slow decline of the specific antibody values was noted in these patients, it cannot be excluded that seropositivity may remain indefinitely, which is often the case with the treponemal tests after treatment for syphilis (17).

Weber et al. (7), by means of an immunofluorescence assay, could document a significant decline of serum IgG antibodies to *B. burgdorferi* after treatment in 18 of 29 patients with ACA, but the details of the follow-up time are not clearly given. By using ELISA, Åsbrink et al. (5) found, in 1984, a significant decrease of specific antibody values in 15 of 26 (58%) patients with ACA 6–13 months after treatment. Our results from the present larger study are thus in agreement with this report.

At the last clinical control, the infection had been clinically judged as cured in all 74 patients. Residual signs or symptoms present in some patients had been considered compatible with sequelae but not likely to represent active infection. After an essential regression of the erythema and of the oedema, residual dilated dermal blood vessels, heel-swelling compatible with organized oedema and persistent atrophy of the skin were accepted as sequelae and not as signs of persistent infection. Active arthritis that could be linked to the borreliosis was not present. As regards polyneuropathy, it has recently been shown that in spite of good results of antibiotic treatment for symptoms of pain and paresthesia, the neurological deficit is usually unchanged at a clinical and neurophysiological follow-up (18). Among the patients in the present study, there was no progress of residual symptoms of polyneuropathy at the last control.

Clinical assessments of the infection as cured also referred to the 6 patients in whom a significant decline of the specific antibody values in serum was not documented during the follow-up. It is possible that further serological controls of these 6 patients would have revealed declining values, but we cannot exclude that a few patients retain stationary or only insignificantly declining values of serum antibodies to *B. burgdorferi* after treatment of ACA, in spite of the absence of clinical indications of persistent infection.

The fact that some patients had received more than one course of antibiotics, in some cases at a time when a significant decrease of the specific antibody levels had already occurred, may to some extent complicate the interpretation of the results. These patients were not excluded from the study, as that would have made it biased. The choice of antibiotic and route of administration in the treatment of the patients included in the present

study had been governed by the severity of the clinical manifestations but also by current knowledge about the borreliosis, which has changed during the years since 1983, when the first patients were enrolled. Today we consider retreatment within 4 months due to persistent cutaneous manifestations, as performed in 2 patients, as unnecessary, since recovery often requires more time. The residual heel-swelling that was the motivation for retreatment in another patient we now interpret as a sequela due to organized oedema and not as a sign of persistent infection. The evaluation of extracutaneous manifestations, such as joint manifestations or polyneuropathy, can be difficult. Several of the patients had received additional antibiotics because of uncertainty about the sufficiency of an initial oral penicillin therapy with regard to extracutaneous manifestations. These patients would today have received intravenous penicillin or oral doxycycline as first choice.

From the present study it cannot be definitively concluded that a significant decrease of the specific antibody values is a proof of cure. A serological comparison between patients with and without obvious treatment failures is needed to answer the question. However, the results of the study show that a significant decline of the levels of serum IgG antibodies to *B. burgdorferi* can be expected in the majority of patients who do not have clinical evidence of persistent infection after antibiotic treatment of ACA. In more than half of the patients, this decline is found within a period of approximately one year. We suggest that these results can be used as guidelines and that serology can be of help in the follow-up after treatment of patients with ACA.

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