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CLINICAL REPORT

Long-term Serological Follow-up of Patients Treated for Chronic Cutaneous Borreliosis or Culture-positive Erythema Migrans

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The kinetics of antibodies to *Borrelia burgdorferi* following successful treatment of early and late cutaneous borreliosis were analysed in consecutive serum samples by an enzyme-linked immunosorbent assay (ELISA) technique. Twenty-three patients with culture positive erythema migrans were followed for 23 ± 14 months: 41% stayed seronegative, 35% showed an isolated immunoglobulin M (IgM) response, 8% an isolated IgG response and 16% a combined IgM and IgG responses. In general, antibody levels peaked within the first 3 months of symptom onset, whereafter a gradual decline was observed within 1 year. Twenty-two patients with chronic cutaneous borreliosis were followed for 23 ± 11 months and all patients stayed IgG positive. Nearly three-quarters showed a clear decline in IgG levels over the years, while the rest did not. After 9 ± 1 years 88% of 16 patients examined were still IgG positive. In conclusion, treatment of erythema migrans should be initiated on clinical appearance as a substantial number of patients stayed seronegative. Treatment success may in part be monitored serologically for both seropositive erythema migrans and chronic cutaneous borreliosis as most patients show declining titres after successful treatment. However, continuously high titres do not necessarily indicate treatment failure.

Key words: acrodermatitis chronica atrophicans; ELISA; Borrelia burgdorferi.

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Borrelia burgdorferi senso lato has been recognized as the tick-borne aetiological agent of Lyme borreliosis (LB). LB is a multisystem disorder progressing in early localized and later disseminated stages potentially affecting the skin, joints, heart and nervous system. The dermatological lesions are the early erythema migrans (EM) and the late acrodermatitis chronica atrophicans (ACA). Along with ACA lesions, late cutaneous borreliosis may show a number of less characteristic manifestations, e.g. scleroderma-like lesions, erythematous ulnar bands and nodules. To include these, the term chronic cutaneous borreliosis (CCB) may be preferred for manifestations with a duration of more than 6 months and this term is presently used. Some excellent reviews have been published on cutaneous borreliosis, histology and immunology (1–5).

The serology of LB may be difficult to interpret and has a number of limitations including: (i) low diagnostic sensitivity in early disease due to a slow and late appearing antibody response; and (ii) antibody persistence making it difficult to distinguish between actual or earlier infection. Thus, in *B. burgdorferi* endemic areas a number of persons with no history of LB shows positive *Borrelia* antibodies (6–8), and antibodies may persist for years in untreated LB (9–11) as well as after successful treatment of EM (12–17). A significant number of patients treated for EM shows no antibodies to *B. burgdorferi* in serum at the time of diagnosis or thereafter (12–15, 18–20). In contrast, almost all late-stage LB patients with CCB have been found to be seropositive at the time of diagnosis and in some patients immunoglobulin G (IgG) antibodies may persist for several years after treatment (15, 17, 21–24). In general, the positive predictive value of LB serology is low if there is little clinical evidence of LB (25).

The diagnosis and treatment of LB are usually based on a combination of clinical, histopathological and serological findings, and subsequently supported by a positive treatment response. As a proportion of patients shows only slow clinical regression after antibiotic treatment it would be valuable to use serology as a supportive means to distinguish treatment failures from postinfectious sequelae. To examine the kinetics of specific antibodies after treatment in patients with EM or CCB the present long-term serological follow-up study was undertaken. Results on 23 patients with culture-proven EM and 22 patients with CCB are reported.

MATERIAL AND METHODS

Patients

A total of 23 patients (12 women and 11 men), aged 52 ± 12 years (mean ± SD), with culture-positive EM were included in the study. Twelve patients were admitted to the Department of Dermatology, Marselisborg Hospital, Aarhus, Denmark, in the period from July to November 1989, and 11 patients were admitted to Department of Dermatology, Odense University Hospital, Odense, Denmark, during 1992. Pretreatment symptom duration was 5 ± 3 weeks. All patients were previously healthy with no other skin disorders. *Borrelia burgdorferi* could be cultured from 41% of the EM patients visiting the departments during the study periods, and to avoid clinical misdiagnosis only the culture-positive patients were included.

Twenty-one patients were treated with oral phenoxy-methylpenicillin 1.5×10^6 IU 3 times a day for 10 days. The remaining 2 patients were initially treated with oral roxythromycin 150 mg b.i.d. for 10 days but treatment was repeated with oral phenoxy-methylpenicillin due to disease relapse with culture-positive biopsies. All patients showed complete regression of the lesions within a few weeks after treatment.

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Twenty-two patients with CCB admitted to the Department of Dermatology, Marselisborg Hospital, during 1986–1990 were included in the study. The diagnosis was based on the combination of: (i) ACA lesions characterized by violaceous discoloration, atrophic skin and varying degrees of pigmentation. Some patients also showed fibrous nodules, erythematous ulnar bands or sclerodermic lesions; (ii) histopathology with plasma cells, dermal lymphocytic infiltrates and sometimes fibrosis; and (iii) the presence of anti-*B. burgdorferi* antibodies. In addition, all patients showed a good clinical response weeks to months after 3 weeks of treatment with either oral phenoxymethyl-penicillin 1.5×10^6 IU 3 times a day or oral doxycyclin 100 mg twice a day. After 1–2 years only slight discoloration and atrophic changes remained. Patient characteristics are shown in Table I.

In vitro cultivation of Borrelia burgdorferi from skin biopsies

From patients clinically presenting with EM a 4 mm punch biopsy was obtained under sterile conditions from the margin of affected skin. The specimen was immediately incubated at 32°C in 7 ml of BSK (Barbour–Stoenner–Kelly) medium without rabbit serum or antibiotics. Cultures were examined weekly by dark-field microscopy and detected spirochetes subcultured and identified as *B. burgdorferi* by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), Western blotting and DNA/DNA hybridization as described previously (26).

Enzyme-linked immunosorbent assay

Serum samples were obtained from all patients prior to treatment and on several occasions thereafter, initially at intervals of 1–2 months. The sera were stored at – 20°C until analysis. Serum antibodies to *B. burgdorferi* flagella were detected using an indirect enzyme-linked immunosorbent assay (ELISA) for IgG (19) and a μ-capture ELISA for IgM as described previously (27). The diagnostic cut-off was defined as the 98th percentile of optical density (OD) values detected by examination of 200 healthy control sera. Consecutive samples from each patient were examined on the same microtitre plate to exclude plate-to-plate variation and a 2-fold change in the ELISA OD value was considered significant.

From most CCB patients a late serum sample was obtained several years after the initial consecutive follow-up period. This sample was analysed separately from initial samples.

Table I. *Clinical data of patients with chronic cutaneous borreliosis*

Number of patients	22
Gender, female/male	17/5
Age (years) (mean ± SD)	47 ± 12
Pretreatment symptom duration	
<2 years	7
2–5 years	8
>5 years	7
Consecutive follow-up time (months) (mean ± SD)	23 ± 11
Late serum sample (years) (mean ± SD)	9 ± 1
Treatment	
Penicillin	12
Doxycyclin	6
Penicillin/doxycyclin	4
Treatment response	
Complete regression	13
Marked improvement	9

RESULTS

Erythema migrans

The 23 EM patients were followed serologically for 23 ± 14 months (mean ± SD).The results are shown in Figs. 1 and 2, with data on the 12 patients diagnosed in 1989 and the 11 patients diagnosed in 1992 illustrated separately since the ELISA assays were run on separate days with different cut-off values. In summary, 9 of the 23 patients (41%) were seronegative during the entire follow-up period, 8 patients (35%) showed an isolated IgM response and 2 patients (8%) an isolated IgG response and 4 patients (16%) showed concomitant IgM and IgG responses.

In general, peak antibody levels were found for IgG 1–5 weeks after diagnosis and for IgM 1–6 weeks after diagnosis, corresponding to peak levels within 3 months of symptom onset. However, 1 patient did not mount an IgG response until 7 months after symptom onset. Following the peak, the IgM response gradually declined, becoming negative or significantly decreased within 1 year (Fig. 1). Notably, one patient showed an exceptionally long-lasting IgM response. Five of the 6 IgG-positive patients became seronegative within 6 months (Fig. 2). The remaining patient with the most pronounced and long-lasting IgG response had been treated 2 years previously with erythromycin for EM and the isolated IgG response therefore probably represented a booster reaction.

Chronic cutaneous borreliosis

The serological response of the 22 CCB patients was followed consecutively for 23 ± 11 months (mean ± SD). The majority

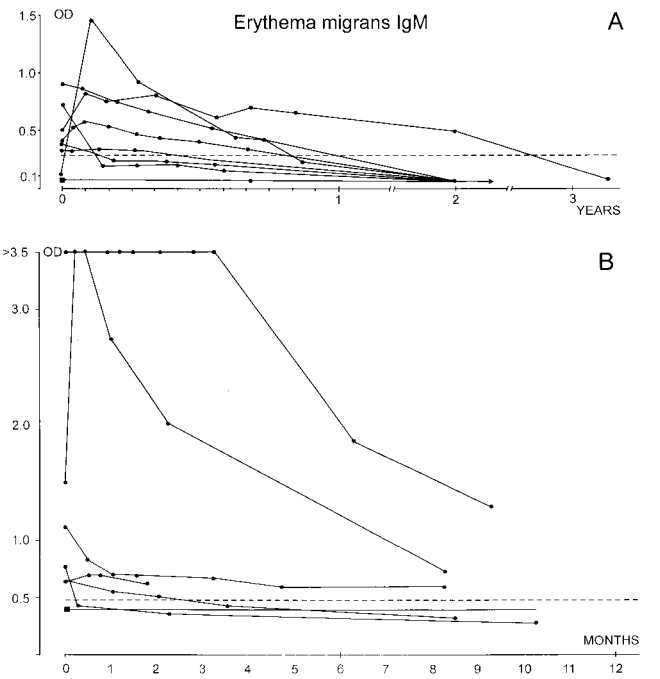

Fig. 1. Optical densities (OD) of IgM against *B. burgdorferi* at the time of diagnosis and during follow-up. (*A*) 12 patients with erythema migrans diagnosed in 1989; (*B*) 11 patients with erythema migrans diagnosed in 1992. The horizontal dashed lines mark the cut-off value. ■, Seronegative patients (A= 5 patients, B= 5 patients).

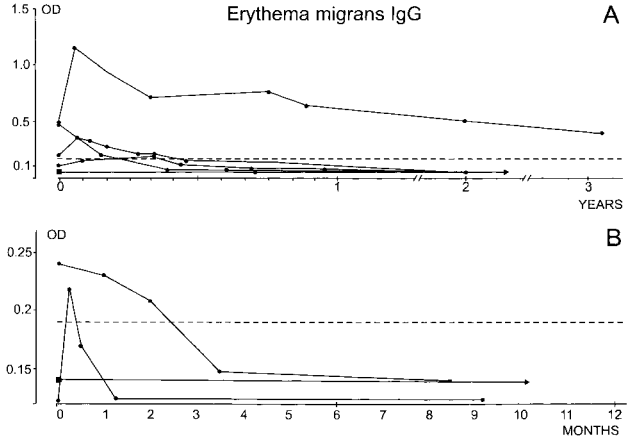

Fig. 2. Optical densities (OD) of IgG against *B. burgdorferi* at the time of diagnosis and during follow-up. (*A*) 12 patients with erythema migrans diagnosed in 1989; (*B*) 11 patients with erythema migrans diagnosed in 1992. The horizontal dashed lines mark the cut-off value. ■, Seronegative patients (A= 8 patients, B= 9 patients).

of pretreatment samples showed high IgG OD values with no correlation to the duration of disease prior to diagnosis. Fig. 3 illustrates the kinetics of the IgG response after treatment in relation to disease duration. For 13/22 patients a significant decline was detected within 1–2 years. Three patients followed for only 8, 8 and 22 months, respectively, showed a clear and almost significant decline which may have become significant with further follow-up. Six patients showed no or only very little decline. Of these 6 patients, 4 experienced a total remission of symptoms while the remaining 2 showed considerable improvement. All 22 CCB patients showed continuously positive IgG during the consecutive follow-up period.

After the initial consecutive follow-up period the CCB patients were controlled clinically and serologically at intervals of 1–2 years. Most patients showed a gradual serological decline and further clinical improvement leaving only a slight discoloration. A late serum sample was obtained from 16 of the 22 CCB patients 9±1 years (mean±SD) after diagnosis and treatment. At this point only 1 patient showed a positive but low OD value in the IgM ELISA. However, 14/16 still showed significant positive IgG values. No clear relationship between disease duration before diagnosis and late follow-up IgG values was detected.

DISCUSSION

Borrelia burgdorferi was cultured from the lesions of all 23 EM patients, thereby excluding the possibility of clinical misdiagnosis and false-positive serology. Such a well-defined patient group is ideal for studying the rate of seroconversion before and after treatment and for following the antibody kinetics after treatment. Only 1 previous study has described a long-term serological follow-up on culture-positive EM patients (14). As for EM, cultivation of *B. burgdorferi* from all CCB patients would be ideal. Unfortunately, this is not feasible from most CCB lesions, possibly because of a low density of spirochetes. As in previous studies the diagnosis of CCB was based on the combination of characteristic clinical and histopathological findings and positive *Borrelia* IgG, subsequently supported by a good response to treatment.

From the 23 EM patients serum samples were collected regularly over an average period of nearly 2 years. During this period 41% stayed seronegative. In comparison with 2 previous studies on culture-confirmed EM patients, this is in good accordance with Strle et al. (28), who reported > 50% seronegative, but in contrast to only 9% seronegative detected by Aguero-Rosenfeld et al. (14). Earlier studies on clinically diagnosed EM patients have shown negative antibody responses in about 20–30% of patients (12, 13, 20). Taken together, a significant proportion of EM patients does not possess serum antibodies to *B. burgdorferi* at the time of

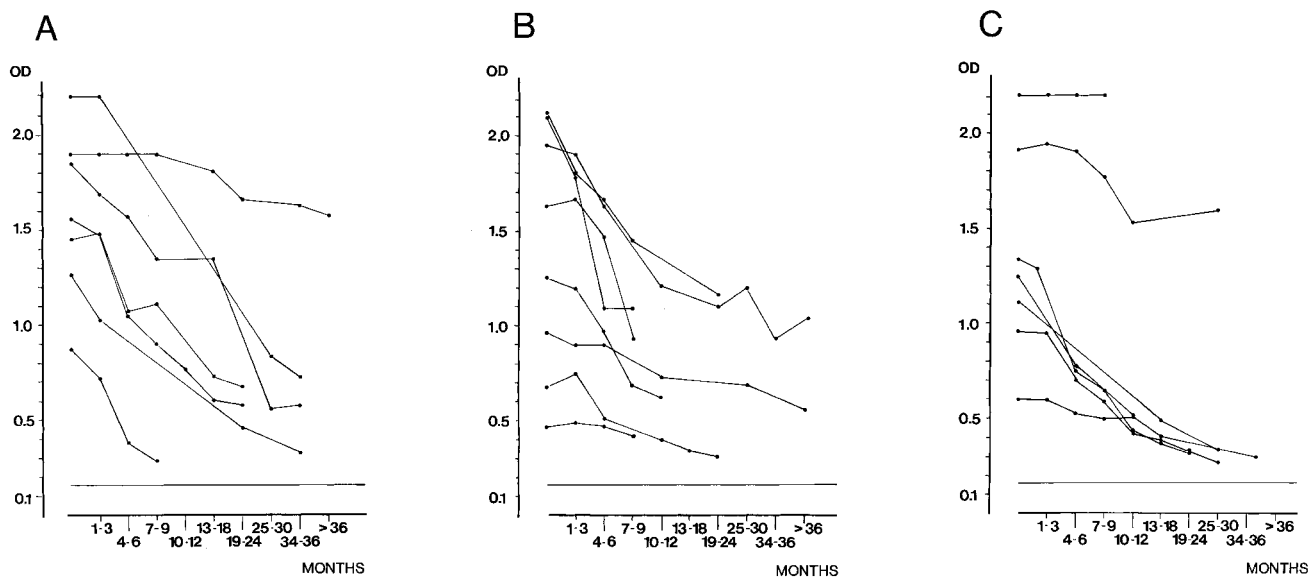

Fig. 3. Optical densities (OD) of IgG against *B. burgdorferi* from 22 patients with chronic cutaneous borreliosis at the time of diagnosis and during follow-up. ODs are illustrated according to disease duration prior to diagnosis and treatment as follows: (*A*) <2 years, (*B*) 2–5 years, and (*C*) >5 years. The horizontal lines mark the cut-off value.

diagnosis, and subsequent to treatment they do not mount a response. Therefore, treatment must be initiated on the basis of clinical findings. It is not known whether these patients potentially would have raised a response which was abrogated by the early treatment. In addition, it may be that spirochetes are cultured more readily from seronegative than from seropositive patients.

Interestingly, only 4 of the 14 seropositive EM patients showed both an IgG and an IgM response. This is in accordance with previous studies (16, 21) which reported the frequent finding of a response in only one of the immunoglobulin classes, most commonly IgM. It may be that early treatment abrogates the normal seroconversion from IgM to IgG antibodies, as previously noted by others (16). In the present study unspecific "flu-like" symptoms were the only extracutaneous manifestations observed, and these were most often recorded for patients with only an IgM response. In a study by Åsbrink et al. (21) EM patients with concomitant extracutaneous manifestations, such as facial palsy, meningoradiculitis and electrocardiographic disturbances, were more often both IgM and IgG positive. The isolated IgG response detected in 2 patients possibly represented a secondary response, although only 1 of the patients presented with a history of previous borreliosis. Alternatively, the second patient did not mount a primary IgM response as these antibodies would be expected to persist at the time of diagnosis at only 8 weeks of disease duration.

All EM patients recovered completely shortly after treatment and showed peak antibody levels at the time of treatment or within a few weeks. With the exception of a few patients, a significant decline in antibody levels occurred within 1 year following successful treatment. Other studies have shown a similar decline for most patients; however, most studies report a somewhat higher proportion of seropositive samples after 1 year, ranging from 16% to 59% (12–16). This discrepancy may in part be explained by the short duration of the IgG response found in the present study. In this context, it has been shown by others that a few EM patients may stay IgG seropositive for at least 10 years (16, 17) and that the IgM response may be long lasting (16).

Most CCB patients showed high OD values on ELISA at the time of diagnosis compared with the EM patients, and none of the patients became seronegative during the consecutive follow-up period of a mean 23 months. In 16 patients a late serum sample was obtained on average 9 years after treatment, and 14 patients still showed IgG anti-flagellum antibodies. This clearly illustrates that such antibodies are present in a considerable number of patients several years after successful treatment and clinical regression, and thereby corroborates and extends the findings of several previous studies (15, 17, 21–24).

During the initial follow-up period approximately three-quarters of patients showed a clear decrease in OD value. However, one-quarter showed no decrease. Thus, it seems that the majority of patients showed a declining antibody level in the years following treatment. However, a lack of decline does not necessarily indicate insufficient treatment. Notably, none of the patients showed increasing titres, which would possibly indicate treatment failure, although the antibody kinetics of untreated CCB is not well known.

In conclusion, treatment success in seropositive EM and CCB patients may in part be monitored serologically as a decline in antibody levels is expected for the majority of patients within 1 year for EM and over several years for CCB patients. The latter may stay seropositive for many years after successful treatment and a continuously high titre does not necessarily indicate treatment failure.

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