ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2004, 11, 145–148

ANTINUCLEAR ANTIBODIES ARE NOT INCREASED IN THE EARLY PHASE OF *BORRELIA* INFECTION

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Śpiewak R, Stojek NM, Chmielewska-Badora J: Antinuclear antibodies are not increased in the early phase of *Borrelia* infection. *Ann Agric Environ Med* 2004, **11**, 145–148.

Abstract: In the literature, there are case reports suggesting that *Borrelia burgdorferi* infection may induce autoimmune diseases dependent on antinuclear antibodies (ANA). The present study was undertaken in order to verify this possibility in a prospective manner. The study group comprised 78 consecutive patients (51 women and 27 men, median age 41.5 years) referred to our Department for the serologic diagnosis of *Borrelia* infection. The patients' sera were tested for *Borrelia*-specific IgM and IgG (Recombinant Antigen Enzyme Immunoassays, Biomedica). Antibodies against *Borrelia* were detected in 31 (39.7%) persons. 15 persons (19.2%) had positive IgM, another 15 (19.2%) - positive IgG, and 1 person (3.2%) - both IgM and IgG. Frequent positivity of IgM antibodies suggests that persons in the early phase of infection prevailed in the group. Tests for anti-dsDNA, anti-RNP, anti-Sm antibodies, and a screening test for systemic rheumatic diseases (ANA Rheuma Screen) were carried out using Varelisa Enzyme Immunoassays (Pharmacia&Upjohn). The spectrum of autoimmune diseases covered by these tests included SLE, MCTD, Sjögren's syndrome, scleroderma, polymyositis, and dermatomyositis. ANA were detected in 15 persons (19.2%): anti-dsDNA in 7 (9.0%), anti-RNP in 1 (1.3%), anti-Sm in 2 (2.6%), and ANA Rheuma Screen was positive in 6 persons (7.7%). Statistical analysis of differences in the ANA frequency between *Borrelia*-positive and -negative groups was carried out using Fisher's exact chi-square test (both without and with gender and age matching). No significant differences were found between the groups. Based on the above results, we conclude that there is no increase in the frequency of antinuclear antibodies in the early phase of *Borrelia* infection.

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Key words: *Borrelia burgdorferi*, borreliosis, Lyme disease, autoantibodies, antinuclear antibodies, anti-dsDNA, anti-RNP, anti-Sm, autoimmune diseases, serological study, Poland.

In the literature, there are casual observations suggesting that *Borrelia burgdorferi* infection may induce autoimmune diseases dependent on antinuclear antibodies (ANA), i.e. antibodies directed against the body's own cell nuclei. As infection by *B. burgdorferi* is common in Poland, this kind of complication would be of great importance to public health. Until now, however, no scientific evidence has been produced for the existence and frequency of such complication. To fill this gap, the present study was undertaken in which we investigated the possible difference in the frequency of antinuclear antibodies between *Borrelia*-infected and non-infected people.

Received: 13 April 2004
Accepted: 20 May 2004

MATERIAL AND METHODS

Study population. A prospective study of consecutive patients referred to our department for the serologic diagnosis of *Borrelia* infection was carried out in autumn and winter 2001/2002. The criterion for inclusion was suspicion of borreliosis, clearly stated by the referring physician. Altogether, 78 persons were tested: 51 women and 27 men aged 9–78 (median 41.5) years.

Serology investigations. The patients' sera were tested for anti-*Borrelia* atibodies using IgM and IgG *Borrelia* Recombinant Antigen Enzyme Immunoassays (Biomedica Medizinprodukte, Vienna, Austria). At the same time, anti-dsDNA, anti-RNP, and anti-Sm antinuclear antibodies were sought in the serum by the means of Varelisa Enzyme Immunoassays, (Pharmacia&Upjohn, Freiburg). Additionally, a screening test for systemic rheumatic diseases was carried out using Varelisa ANA Rheuma Screen which includes the following nuclear antigens: dsDNA, histones, U1-snRNP, Sm, RNP-Sm, SS-A/Ro, SS-B/La, Scl-70, centromere, Jo-1, and PM-Scl-100. According to the manufacturer's information, the spectrum of autoimmune diseases covered by the serology tests includes SLE, MCTD, Sjögren's syndrome, scleroderma, polymyositis, and dermatomyositis. For all tests carried out, the qualitative results were interpreted according to the product information. In further statistical analysis, equivocal results were considered negative.

Statistical analysis. Depending on the positivity of *Borrelia* antibodies, the study subjects were divided into two groups denominated as *Borrelia*(+) and *Borrelia*(–) respectively. The frequencies of antinuclear antibodies in both groups were compared using Fisher's exact chi^2 test. As a second step, the gender and age matching of the study persons was carried out in order to avoid a possible bias caused by these factors [1, 22]. Groups consisting of the matched subjects were denominated as $Borrelia(+)_{match}$ and $Borrelia(-)_{match}$. T-test for independent samples, Kruskal-Wallis test and the median test were used to compare age structures in the matched groups; none of these tests was capable of showing significant age difference. After the matching, the frequencies of antinuclear antibodies were re-analysed in matched groups using Fisher's exact chi^2 test (SPSS, Statsoft, Tulsa, USA).

RESULTS

Among 78 persons included into our study, antibodies against *Borrelia* antigens were detected in 31 (39.7%). 15 persons (19.2%) had positive IgM, another 15 (19.2%) - positive IgG; one person (3.2%) was both IgM- and IgG-positive. ANA were detected in 15 of the 78 persons (19.2%): anti-dsDNA in 7 (9.0%), anti-RNP in 1 (1.3%), and anti-Sm in 2 (2.6%). Varelisa Rheuma Screen was positive in 6 persons (7.7%). The frequencies of antinuclear antibodies in both groups are shown in Table 1. The gender and age matching produced 12 pairs of women (median age for negative group 46 years, for positive group 45 years) and 10 pairs of men (for both groups median age was 37 years). The results for the matched groups are presented in Table 2. No significant differences were found both before and after matching.

Table 1. Antinuclear antibodies in *Borrelia*-positive and negative groups: results before gender and age matching.

	N	ANA positive	anti-dsDNA positive	anti-RNP positive	anti-Sm positive	Rheuma Screen positive
Borrelia(+)	31	5 (16%)	2 (6%)	0	1 (3%)	2 (6%)
Borrelia(–)	47	10 (21%)	5 (11%)	1 (2%)	1 (2%)	4 (9%)

Table 2. Antinuclear antibodies in *Borrelia*-positive and negative groups: results after gender and age matching.

	N	ANA positive	anti-dsDNA positive	anti-RNP positive	anti-Sm positive	Rheuma Screen positive
$Borrelia(+)_{match}$	22	5 (23%)	2 (9%)	0	1 (5%)	2 (9%)
$Borrelia(-)_{match}$	22	6 (27%)	4 (18%)	0	0	3 (14%)

DISCUSSION

Infection by *Borrelia burgdorferi* - a spirochete bacterium causing borreliosis (Lyme disease) is relatively common in Poland. Serological evidence of present or past *Borrelia* infection was found in 11–15% farmers in eastern Poland [4, 8] and in as many as 61% forestry workers in north-western Poland [25]. The main vector of the infection is *Ixodes ricinus* tick. A correlation has been observed between the tick activity and incidence of infection in exposed people [6]. *Borrelia* spirochetes could be detected in 8.8–13.2% of the ticks [3, 7, 31, 32, 40]. Other arthropods are also likely to transmit the infection, for example *B. burgdorferi* could be detected in up to 3.2% mosquitoes [21]. The situation in neighbouring Slovakia and Czech Republic is comparable to that in Poland [9, 41]. Domestic and wild animals form a large reservoir of the infection. For example, seropositivity to *B. burgdorferi* was found in 40% of dogs from endemic areas in Poland [29, 30], 17% of sheep and 19% of goats in Slovakia [37], and up to 60% of wild rodents in northern Czech territory [38].

Facing the high frequency of *Borrelia* infections, their immunological complications pose a serious threat to the public health. *Borrelia* was demonstrated to trigger a range of autoimmune reactions, i.e. reactions in which body's immune system turns against its own structures. The well-documented examples are inflammatory diseases of the central nervous system (CNS) and Lyme arthritis. In the CNS, *Borrelia* infection triggers an

Table 3. Overall frequency of antinuclear antibodies in the present study compared to other eastern-Polish populations (95%-confidence intervals are given in brackets).

	N	anti-dsDNA (%)	anti-RNP (%)	anti-Sm (%)	Rheuma Screen (%)	ReCombi ANA Profile* (%)
Present study	78	9.0 (2.6–15.3)	1.3 (0.0–3.8)	2.6 (0.0–6.1)	7.7 (1.8–13.6)	NT
Blood donors [34, 35]	50	2.0 (0.0–5.9)	2 (0.0–5.9)	0	NT	12.0 (3.0–21.0)
Mixed population [36]	130	9.2 (4.3–14.2)	4.6 (1.0–8.2)	1.5 (0.0–3.7)	NT	NT
Rural inhabitants [34]	90	12.2 (5.5–19.0)	5.6 (0.8–10.3)	2.2 (0.0–5.3)	NT	30.0 (20.5–39.5)

NT - not tested; *ReCombi ANA Profile covers 8 of 11 antigens present in Rheuma Screen - the results of both tests may be considered as comparable, though not identical.

autoimmune reaction that leads to demyelination of the nervous tissue [23]. Recently, an increased frequency of *Borrelia* antibodies among patients with multiple sclerosis was reported [5]. Lyme arthritis is an autoimmune reaction against human leukocyte function-associated antigen-1 (hLFA-1) which is cross-reactive with outer surface protein A (OspA) of *B. burgdorferi* [14, 15]. In this way, antibodies produced in order to destroy the invading bacteria bind and initiate damage to the body's own structures [28].

In contrast to the above-mentioned diseases, it remains unclear whether *Borrelia* infection is capable of inducing antibodies directed against the body's own cell nuclei (ANA). There are a handful of case reports on the co-existence of *Borrelia* infection and ANA-related autoimmune diseases, such as dematomyositis [2, 12, 17, 19], systemic lupus erythematosus (SLE) [11] and generalized morphea [24]. Moreover, histopathologic similarities between borreliosis and SLE were pointed out [10, 16], and a raised titre of ANA in the course of borreliosis was reported [13, 26]. On the other hand, false-positive reactions to *Borrelia* antigen were described among patients with SLE [20, 39].

In the present study, we were not able to confirm any relationship between the presence of *Borrelia* antibodies and ANA. Of 31 *Borrelia*-positive persons, IgM-class antibodies were detected in 16, which indicates an early stage of the infection. It cannot be excluded that autoimmune reactions might start only in later stages, bearing in mind that *Borrelia*-related immune disturbances may persist over 10 years after infection [33]. A comparative study of patients with progressed borreliosis would shed additional light on the topic.

As shown in Table 3, the overall ANA-positivity rates among persons participating in the present study (both *Borrelia*-positive and -negative) were higher than among eastern-Polish blood donors studied previously [34, 35], which may result from the fact that all the blood donors were males. The frequency of ANA in males is lower than in females, possibly due to the lower estrogen levels [1]. Present results were most comparable to data from another random eastern-Polish population [36]. At the same time, the observed rates were lower than those seen previously among rural inhabitants [34]. Increased frequencies of ANA among rural residents are attributed to their long-term exposure to pesticides [18, 27, 34].

CONCLUSION

Based on the above results, we conclude that there is no increase in the frequency of antinuclear antibodies in the early phase of *Borrelia* infection.

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