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Longterm survey (7 years) in a population at risk for Lyme borreliosis: What happens to the seropositive individuals?

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Abstract. In 1986, a 26% seroprevalence of IgG- anti-*Borrelia burgdorferi* antibodies was observed among 950 orienteers and the incidence of new clinical infections was 0.8%. In 1993, a total of 305 seropositive orienteers were reexamined. During that time, 15 cases (4.9%) of definite/probable Lyme disease occurred in this seropositive group (12 skin manifestations and 3 monoarticular joint manifestations). Among the 12 definite cases, 9 showed new clinical infections (7 EM, 1 acrodermatitis chronica atrophicans, 1 arthritis), and 3 were recurrent (2 EM, 1 arthritis). The annual incidence (0.8%) in this seropositive group was identical to the incidence observed among the whole population in 1986. The individual antibody titer decreased slightly but the seroreversion rate was low (7%). Serology was not very helpful in identifying clinical cases and evolutions, and it can be stated, that a positive serology is much more frequent in this risk group than clinical disease.

Key words: Longterm survey, Lyme borreliosis, Orienteers, Population at risk

Introduction

Lyme borreliosis (LB) is now a well-known disease entity, caused by the spirochete *Borrelia burgdorferi* (Bb) [1, 2]. This spirochete is transmitted to man by the bite of arthropodes, in Europe by the tick *Ixodes ricinus* [3].

The disease may first appear in its most frequent, but not obligatory form of a ringlike skin lesion called Erythema (chronicum) migrans (EM), a usually harmless manifestation, but pathognomonic for the identification, since serology is still not sufficiently reliable and possible later symptoms such as oligoarticular arthritis or radiculitis are clinically not specific enough to confirm the diagnosis.

In 1986, we started a long-term prospective study on Swiss orienteers, who run frequently through wooded areas and bushes, the preferred habitat of *I. ricinus* [4]. Hence, orienteers are highly exposed to tick bites. *I. ricinus* ticks live all over Switzerland, excluding alpine zones, and are infected at a rate of 5–50% [5]. However, data about the seroepidemiology and the clinical prevalence and incidence of LB in general populations living in endemic areas and in groups with elevated risk for infection are sparse, particularly with regard to the long-term aspect of silent and manifest infection [6–8].

The first part of the study began in spring 1986 and was conducted until spring 1988 (phase I) [9, 10], including repeated controls of the whole population. Most remarkable was the discrepancy observed between the high frequency of tick bites and seropositive individuals on one hand, and the relative lack of actual clinical LB, on the other hand. This was in contrast to earlier studies from the USA and encouraged us to a longer follow-up [11, 12]. In the present study (phase II), we focused on the subgroup of seropositive participants in phase I of the study (1986–1988). The idea was that within this seropositive population we would probably find the highest rate of any serological or clinical evolution.

Methods

Study group

In the summer of 1993 a letter was sent to those participants of the phase I study who had presented a positive antibody titer to Bb in at least one sample, asking them to participate once again in this follow-up. A tube for blood sampling was included in this letter as well as a questionnaire referring on the development of medical events since the end of the phase I study, especially observations of EM (exactly described in

the questionnaire), joint inflammation or manifestations of the nervous system, and on the frequency of tick bites. Depending on the date of entry into the study (1986 or 1987), the follow-up time was 6 or 7 years for any of the participants. Telephone or personal contact was maintained until 1996 with a few of the runners, giving a final follow-up time of ten years. The participants were asked to report to their physician for blood sampling and to send both questionnaire and sample to our laboratory at the Neuchâtel University (in phase I of the study blood sampling was done collectively at large national orienteering competitions).

Evaluation of questionnaires

The questionnaire was similar to those distributed in phase I [9, 10] and we anticipated a good knowledge of the disease manifestations among the participants, since they had been informed through various publications and posters during phase I of the study (1986–1988). They had also the possibility to call us in case of any uncertainty.

Two of us (H.F., M.J.S.) made telephone calls to any of the participants who had mentioned development of symptoms during this 6–7 year period. In case of doubts concerning the degree of probability of a symptom being caused by Bb, we contacted the treating physician or requested for a medical report. Our criteria of evaluation corresponded to those of phase I: definite/probable/possible [9]. The definition of EM was a growing ringlike or patchy erythema of at least 3 cm diameter. A symptom was deemed to be definite, when confirmed by a physician or by a telephone interview by us with a high degree of probability. Our judgement was not influenced by the serologic result, which was not yet available at that time.

Enzyme linked immuno sorbent assay (ELISA)

The ELISA sonicate antigen (strain B31) was prepared as described by Russell et al. [13] and the ELISA was performed as previously described [9]. Microtitre plates (DYNATECH, USA) were coated overnight at 4 °C with 50 µl of sonicate antigen diluted in 0.1 M carbonate buffer (pH 9.6). The optimal coating concentration was 6.5 µg of protein/ml. All serum samples were tested in duplicate at a dilution of 1:200. Antibody-antigen reactions were visualized with peroxidase conjugated goat anti-human IgG (GaHu/IgG (Fc)/PO, Nordic) and ortho-phenyldiamine as substrate. In each assay, a negative serum and a strongly positive one (diluted 6 times from 1:300 to 1:9600) were included. The curve derived from the result with the positive serum was used as reference. The optical density (OD) of each serum was compared with the positive control curve, and the result was expressed as the logarithm of the dilution (log dil) of the positive serum corresponding to this OD. The positive cut-off level for IgG antibodies was defined as a log dil < 3.74 or > 122 units which corresponded to 2 standard deviations below the mean log dil of 51 sera of people living at high altitude, in a non endemic area. At this cut-off level, the specificity of the test is 96% if other spirochetes such as leptospires and treponemes were excluded. The sensitivity of the serologic test varied from 26% to 100% depending on clinical manifestations in cases of definite Lyme borreliosis [9]. A significant increase or decrease in the antibody level corresponds to 37 units. Four categories were distinguished according to the antibody level: low: 122–140 units; medium: 141–180 units, high: 181–220 units and very high: >221 units. The sera from the first and second blood samples were tested simultaneously in the same ELISA.

Statistical analysis

The Fischer's test was used to compare LB incidence in the seropositive population and in the high and very high seropositive population (significant difference: $p < 0.05$).

Results

Age and sex distribution

Out of 394 participants who were seropositive during phase I and who were asked to participate in phase II, 305 sent a blood sample and the questionnaire, corresponding to a response rate of 77%. Among these 305 volunteers, 160 (52%) were men, 145 (48%) were women, and the mean age of the whole population was 42 years (10–70).

Table 1. Overview of clinical symptoms of Lyme borreliosis in 305 seropositive individuals from 1986–1988 (phase I) to 1993 (phase II)

Localization of the symptoms	Claimed by orienteers	Assessed probability of LB			
		Definite	Probable	Possible	Negative
Skin	31	10	2	2	17
Joint	29	2	1	5	21
Nervous system	6	0	0	1	5
Total	66	12	3	8	43

Tick exposure

Tick bites during phase II were reported by 247/305 competitors (81%): 112 volunteers (36.7%) recalled between 1–5 tick bites, 76 (24.9%) between 6–10, 54 (17.7%) between 11–50 and 4 (1.3%) even over 50. Absence of tick bites was mentionned by 45 participants (14.8%), and 14 (4.6%) could not remember.

Clinical data

Within the last 6–7 years, 66/305 (21.6%) competitors complained of symptoms: 31 (10.2%) symptoms of the skin, 29 (9.5%) of joints and 6 (2%) of the nervous system (Table 1). Among the 66 participants who reported symptoms, 43 (65%) were unlikely to have Lyme borreliosis symptoms according to our criteria (see Methods). They had skin manifestations such as local tick bite reactions, urticaria, eczema, or joint manifestations due to overuse or traumatic pathogenesis, but not true arthritis. It was also obvious that reported 'neurological symptoms' were mostly manifestations of common pain syndromes due to mechanical problems of running. The number of orienteers with definite/probable LB was reduced to 15/305 (4.9%): 12 cases with skin symptoms and 3 with joint symptoms (Table 1). Among these 15 symptoms, 12 were judged as 'definite' LB, including 9 EM, 1 acrodermatitis chronica atrophicans (ACA) and 2 cases of monoarthritis of the knee. The remaining 3 cases were judged as 'probable' LB, namely 2 EM and 1 arthritis. This gonitis (orienteer no 28, Table 2) was first assessed to be from 'mechanical origin', based on the surgeon's arthroscopy report describing chondropathic lesions beside the synovitis. However, the positive answer to a ceftriaxon treatment and the very high titer of antibodies (310 units) developed by this woman made this case to a probable LB. This was the only case where the serological result was critical for the diagnosis. Finally, 8 cases were classified as 'possible' LB (2 EM, 5 joint and 1 neurological symptoms) (Table 1).

This results in a final disease rate ('definite and probable') of 4.9% for the whole subgroup and hence in an annual incidence of approximately 0.8% (corresponding to the mean follow-up time of 6.5 years)

Description of cases

a) Definite/probable cases of LB. Among the 12 definite cases, 9 were clearly first occurrences of LB (Table 2). All of them presented antibodies in the first part of the study, but had been symptom-free until 1988, and clinical manifestations only appeared during the follow-up between 1988 and 1993 (phase II). A significant decrease in the antibody titer of more than 37 units occurred in one individual (no. 74) whereas 5 other orienteers showed a non signifcant decrease in antibody titer in the follow-up period (5/5 ended by seroconverting to negative values), 2 remained stable (nos. 79 and 312). Only 1 (no. 192) had a slightly higher antibody titer in 1993. All of the 9 orienteers received an antibiotic treatment between 1988 and 1993.

Table 2. Definite and probable Lyme borreliosis cases developed during 1986–1993 by orienteers who participated in phase II of the study

Case no.	Age	Sex	Antibody titer[a]		Symptoms		Treatment
			1986/1988	1993	Before 1988	1988–1993	
2	23	f	254	133	EM 1987	EM 1993	Megacillin
3	56	f	201	133	Arthritis 1983–1988	Arthritis 1989	Ceftriaxon
24	34	f	126	105	–	EM 1992	Doxycycline
32	47	f	126	121	–	EM 1989	Doxycycline
37	69	m	129	103	–	EM 1992	Doxycycline
74	69	m	177	133	–	EM 1992	Penicilline
79	65	m	123	127	–	EM/Arthralgia	Doxycycline
170	66	m	141	117	EM 1987	Recurrent EM	–
176	47	m	126	118	–	Arthritis 1990	Ceftriaxon
192	28	f	134	144	–	EM 1992	Penicilline
200	47	m	133	110	–	EM 1988	Doxycycline
312	32	f	198	211	–	ACA 1993	Doxycycline
28	51	f	281	310	–	Arthritis 1993	Ceftriaxon
181	38	m	137	118	–	Recurrent EM	–
205	48	m	136	126	–	Recurrent EM	–

[a] Mean value of the titers obtained in phase I study and when retested in 1993.

EM: Erythema migrans; ACA: Acrodermatitis chronica atrophicans; –: negative.

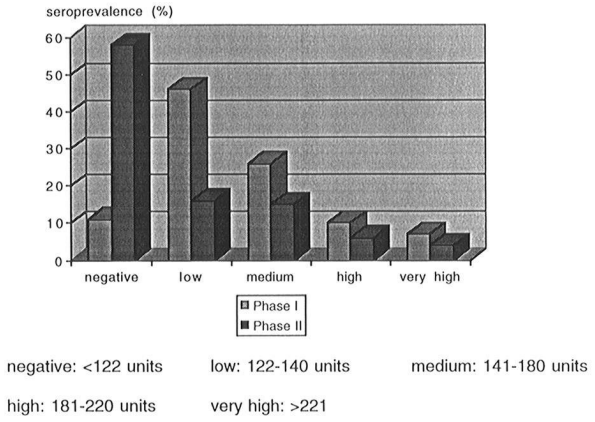

Figure 1. Distribution of the mean values of antibody titers of the sera collected in phase I and of antibody titers of sera collected in phase II.

Among these 9 individuals, only one (no. 312), a female competitor with ACA, presented a persistent high titer in spite of a treatment with doxycycline (198 and 211 units, respectively). One man (no. 176) who had been only slightly positive in 1987 (126 units), was hospitalized for an arthritis in 1990 with strongly elevated titer in immunofluorescence (IgG 1:1024, tested in another laboratory), and was again negative in 1993 (118 units) after the administration of ceftriaxon.

The other 3 definite cases (nos. 2, 3 and 170) had to be considered as recurrent manifestations or reinfections (1 arthritis, 2 EM) (Table 2). The first (no. 2) had a first EM manifestation in 1987 with a clear decrease in the antibody titer, then a second manifestation in 1993. This orienteer was given megacillin by her physician. The antibody titer decreased between 1987 and 1993 from 254 to 133 units, but surprisingly the 1993 value of our study was measured before the treatment! The second definite case (no. 3) had a primary attack of gonarthritis in 1983, then a recurrence in 1985 when a positive Lyme titer was found for the first time by her general physician, but no antibiotic therapy was administered. In the late fall of 1988, a third episode of synovial effusion developed with a negative bacteriological culture (not done for Bb) and a high antibody titer. Following intravenous treatment with ceftriaxon (3 weeks, 2 g daily) no further recurrence occurred until 1993. The titer decreased from 201 in 1987 to 133 units in 1993. The third case (no. 170) developed a recurrent EM almost at yearly intervals, but never showed other signs of the disease and he was never given antibiotics. The antibody titer decreased from 141 to 117 units.

b) Possible cases of LB. The 8 'possible' LB cases are presented in Table 3. They all belonged to the group with particularly high titer (>200 units). One of them (no. 274) had in 1987 a clear seroconversion from 94 units to 228 units and he reported neurological symptoms. However, in the absence of a medical report these symptoms could only be classified as 'possible meningitis'.

Serology

All orienteers (n = 305) who agreed to participate in the second part of the study were seropositive by definition during phase I of the study. A total of 297 blood samples from phase I of the study were available for retesting in parallel with samples from phase II of the study and therefore only these orienteers have been considered here. In these 297 subjects, the distribution of the antibody titers in phase I of the study was the following: 40% (118/297) participants presented low antibody level (122–140 units), 38% (112/297) had medium antibody level (141–180 units), 16% (46/297) presented high antibody level (181–220 units) and 7% (21/297) had very high (>221 units) antibody levels.

Sera collected in phase I of the study were retested in 1993 and the mean value of results obtained in phase I and in 1993 was used to compare the evolution of the antibody titers for each participant between phases I and II of the study. The distribution of the mean values of the sera collected in phase I in the various categories was the following: 34/297 (11%) were negative, 136/297 (46%) had low antibody levels, 76/297 (26%) medium antibody level, 31/297 (10%) high and 20/297 (7%) very high antibody levels (Figure 1).

The distribution of the antibody titers of the sera collected in phase II of the study was the following: 173/297 (58%) of the sera were negative, 48/297 (16%) had low antibody titers, 44/297 (15%), 19/297 (6%) and 13/297 (4%) had medium, high and very high antibody titers, respectively (Figure 1).

A total of 20/297 (7%) individuals presented a seroreversion from positive antibody titers in phase I of the study to negative in phase II, with a significant decrease in the antibody titer of 37 units.

Particular serological events

a) Orienteers with titer increase. Twelve runners had a remarkable titer increase (of 37 or more units) between phases I and II, the maximal increase being of 102 units in a symptomless young male. Among these 12 orienteers, 8 had several tick bites (6–50) during that time, 3 had no bites, one did not reply to the question. Lyme disease related symptoms were denied by 10 of the 12; two mentioned episodes of shoulder and back pain, respectively, but of obvious mechanical character. Since they were seropositive years before, these 12 cases may not be taken as true seroconversions, but the titer increase may be due to a booster effect.

b) Orienteers with high antibody titers. Among the individuals with a particularly high titer (>200 units) in phase I (n = 53/305; 17.4%), fifteen showed definite/probable (n = 7) or possible (n = 8) manifestations of LB in the past (before 1986) (Table 3). This corresponds to a lifetime prevalence of 15/53 (28.3%) within this group. More conclusive is the incidence between 1987 and 1993 (Table 3): eight cases (4 definite/probable, 4 possible) were recorded during this period, resulting in an incidence of 7.5% (4/53) of definite/probable cases within this subgroup, compared to the incidence of 4.3% (15/305) in the whole seropositive population during the same interval. The difference between the two incidence rates was not significant ($p = 0.5$, Fischer's Test).

Discussion

The aim of this prospective study was to obtain information about disease frequency and serological status in a population at risk for LB not only in a cross-sectional but in a longitudinal way. From several studies on the seroprevalence of anti-Bb antibodies in forestry workers, farmers and cross-country runners it was evident, that antibodies may be found in a very high range within populations highly exposed to tick bites [8, 14–17]. On the other hand the prevalence of clinical disease seems to be relatively lower. This was the main finding of phase I of the study on Swiss orienteers started in 1986: a 26% prevalence of IgG antibodies and only 0.8% disease incidence were observed between spring and fall which corresponds to the tick season activity [9]. Disease incidence remained below 1% during the phase I study (spring 1986–spring 1988) [10]. A study on orienteers in Sweden demonstrated positive antibodies in 9% and LB symptoms in the past in 6%, thus showing less discrepancy between serological and clinical prevalences than observed in our study [18]. Findings similar to ours were reported in a Swiss study on forestry workers. A seroprevalence of 35% was shown with a retrospective clinical prevalence of 3.5% over the last ten years, near to our figures (3% lifetime prevalence) [14].

Table 3. Prevalence and incidence of definite/probable and possible Lyme borreliosis cases among runners presenting high antibody titer (>200 units) in phase I (n = 53)

Patient no.	Definite/probable LB (n = 7/53, 13.2%)	Patient no.	Possible LB (n = 8/53, 15.1%)
2	EM 1987/1993[a]	34	Neuritis? 1987
3	Arthritis 1983–1989[a]	51	Arthralgia 1992[a]
28	Arthritis 1993[a]	60	Polymyalgia rheumatica 1991[a]
63	EM 1986	83	Meningitis? 1966, ACA 1993[a]
234	Facial palsy 1986	241	Meningitis? 1972?
244	EM 1986	272	EM? 1992[a]
312	ACA 1993[a]	274	Meningitis 1987
		297	EM? 1983

? Diagnosis not confirmed, reported in patients history.
[a] Occurrence during follow-up 1986–1993.

An interesting and, to our knowledge, not yet completely solved question is, whether anti-*B. burgdorferi* antibodies characterize a past infection without any additional significance or if they represent a silent infection with the potential of later clinical eruption, or, in contrast, a long-lasting protection against the disease. We tried to address this question by specifically following the seropositive subpopulation over a period of 6–7 years. Although the results do not definitively answer the above questions, they do show that the incidence of 4.9% or 0.8% per year among the seropositive runners during the follow up is neither higher nor lower than the 0.8% incidence during the first six-months-period of the study, which resulted from the complete population including seronegative *and* seropositive individuals. The annual clinical incidence during the initial two-years survey from spring 1986 to spring 1988 was also below 1% [10].

Of course these low frequencies in a risk population could be explained by the underreporting of symptoms by the participants. We do not think it was the case since orienteers in Switzerland are well informed about LB and very interested in LB. This is confirmed by the high participation and response rates we had in the different periods of the study. The other explanation could be that we underestimated the symptomatology, because our analysis excluded two thirds of the reported symptoms as being unlikely LB symptoms. It is evident that the final incidence rate is entirely dependent on the assessments made by the treating doctors and us, and only based – with one exception, mentioned above – on clinical facts. So it is not totally excluded that we might have been too strict in eliminating definite/probable disease cases in order to respect the diagnostic criteria established in the first part of the study [9, 10] or because of the difficulty of analysing retrospectively reported cases.

Among the twelve runners presenting a clear titer increase between phases I and II there was no case with clinical symptomatology. Moreover, among orienteers who had high antibody titers in phase I, the incidence of clinical manifestations was not significantly higher than among the whole group of the 305 seropositive individuals. In contrast, in a recent study in a highly endemic area, the symptom 'arthralgia' was found to be significantly more frequent in the sero-

positive group (n = 90, 38% arthralgia), than in the seronegative group (n = 390, 12%) [19].

Our observed incidences in the whole orienteers group are in clear contrast to the Swedish study conducted by Gustafson et al. [20] in an endemic area for Lyme disease, where higher annual incidences of 4.6% and 3.2%, respectively, were found in a two-year survey. However, our results confirm the conclusion drawn in the 'orienteers' study conducted by the same authors, that 'there was no indication that frequent or severe manifestations of LB are common among orienteers in Sweden' [18].

With regards to the clinical severity, most of the cases among our cross-country runners were mild and usually early manifestations which responded well to antibiotic treatment. Even the three arthritis cases did not turn to a serious evolution, though one of them had a recurrent character, but is now completely healed since 7 years.

In accordance with the above mentioned Swedish two-year survey we observed that without any doubt reinfections or reactivations may occur in spite of an earlier episode of LB or a previous elevated antibody titer. We saw in our follow-up survey of seropositive individuals 9 definite cases of a first occurrence, and 3 definite cases of recurrence of the disease. It is very difficult to conclude, whether these cases were just recurrent manifestations of the preexisting disease or true reinfections, or more interestingly 'early manifestations' occurring very late. This would of course modify the concept that the infection usually starts with EM and is followed by antibody production some time later.

In many individuals the antibody titer slightly decreased (non significant antibody unit decrease) over time, leading to a lower seroprevalence, an unexplained phenomenon so far. However, a low number of orienteers (7%) showed a significant decrease in antibody units leading to a seroreversion between phases I and II of the study, in contradiction to results obtained among outdoor workers in the USA where 23% to 53% of seropositive seroreverted [17]. This could be due to the fact that *I. ricinus* ticks in Switzerland are infected by at least four different *Borrelia* genospecies and that one or other of these species might induce long lasting antibody production [21].

Again, serology was not very helpful in identifying clinical cases and evolutions of LB, and it can be stated that a positive serology is much more frequent in this risk group than clinical manifestations. It seems that antibody titers remaining positive over years may neither protect against nor predispose for later manifestations of LB.

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