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ORIGINAL ARTICLE INFECTIOUS DISEASES

Testing patients with non-specific symptoms for antibodies against *Borrelia burgdorferi* sensu lato does not provide useful clinical information about their aetiology

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Abstract

The aim of this study was to determine whether patients with antibodies against *Borrelia burgdorferi* sensu lato or who report a history of erythema migrans (EM) or tick bite are more likely to have non-specific symptoms such as musculoskeletal pain, fatigue, sensory disorder, and headache. The study group comprised 423 subjects with non-specific symptoms tested for antibodies against *B. burgdorferi* sensu lato between July 2012 and December 2014 because of suspicion of Lyme borreliosis (LB). Of these, 285 were females (67%) and 138 were males (33%); the median age was 53 years (range, 7–89 years). Patients with a confirmed diagnosis of LB and patients with a known underlying disease that could influence the development of the symptoms were excluded from the evaluation. Subjects were assigned to the seronegative group or to one of three seropositive groups, and the history of EM and tick bite was also recorded. Statistical analysis was performed with single chi-square tests of independence and multiple logistic regression models. No differences in the occurrence of non-specific symptoms were observed between patients grouped according to antibody status. A history of EM showed no significant effect on any of the non-specific symptoms. A history of tick bite was weakly correlated with joint pain and joint swelling ($p < 0.05$). In conclusion, it is highly unlikely that the complaints are related to a previous infection with *B. burgdorferi* sensu lato. The results show that testing patients with non-specific symptoms for antibodies against *B. burgdorferi* sensu lato in the everyday clinical setting does not provide any useful information about their aetiology.

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Introduction

Lyme borreliosis (LB) is a tick-borne disease caused by certain species of tick-borne spirochetes of the *Borrelia burgdorferi* sensu lato complex. The disease is characterized by a well-known clinical course, effective antibiotic treatment, and laboratory testing that is highly sensitive in the later stages of the disease [1,2]. Nevertheless, in public discourse, LB is often presented in the opposite way, and so-called 'chronic Lyme disease' has become a widely used term in connection with unexplainable clinical conditions potentially leading to disability or even to life-threatening outcomes, even if the aetiological role of infection with *B. burgdorferi* sensu lato is not proven [3–5]. Long-lasting and repetitive antibiotic treatment is widely used, despite the risks and lack of efficacy [6].

The aim of this study was to determine whether patients who have antibodies against *B. burgdorferi* sensu lato or who report a history of erythema migrans (EM) are more likely to have non-specific symptoms, including musculoskeletal pain,

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fatigue, sensory disorder, and headache. The detection of antibodies in serum and a history of EM were used as a surrogate parameter for previous infection with *B. burgdorferi* sensu lato. The data of a high number of individuals were statistically analysed. Previous tick bites were also evaluated for a possible role in this respect. The study was approved by the ethical committee of the Medical University of Vienna.

Patients and methods

The retrospective selection of the study population is shown in Fig. 1. In total, 705 patients were tested for antibodies against *B. burgdorferi* sensu lato between 1 July 2012 and 31 December 2014 at the Institute for Hygiene and Applied Immunology, Medical University of Vienna. The centre offers consultations combined with laboratory diagnosis to patients with suspicion of LB or those with inconclusive antibody results. Patients were referred by other physicians or were self-referred. All were seen by the same physician. Each visit was documented with special respect to symptoms, history of physician-diagnosed EM, including treatment, and history of tick bite. Other known diseases were also assessed. Serological testing included an ELISA for IgG and IgM (*Borrelia*-ELISA; Medac, Hamburg, Germany) and an immunoblot (Anti-*Borrelia* Euroline Westernblot; Euroimmun, Lübeck, Germany) for confirmation of borderline and positive ELISA results. The tests were performed according to the manufacturers' instructions.

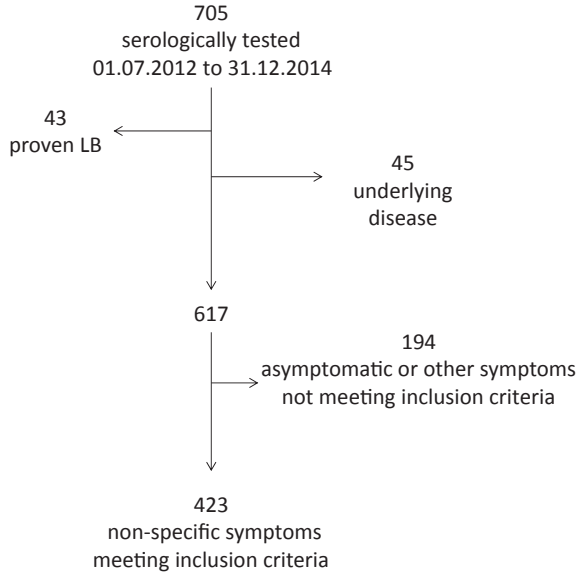

FIG. 1. Flow chart showing selection of the study population. A detailed explanation is given in 'Patients and methods'.

Forty-three patients with proven LB at presentation were excluded from further evaluation: 22 patients with EM, two with multiple EM, five with acrodermatitis chronica atrophicans, three with Lyme neuroborreliosis (LNB) confirmed by cerebrospinal fluid pleocytosis and a positive cerebrospinal fluid/serum antibody index, and five with Lyme arthritis; six patients who had been diagnosed with LNB in the past were also excluded. Forty-five patients presented with a known underlying disease that could have an influence on the symptoms, and these patients were also excluded from the evaluation. Eight of them suffered from a neurological disease, including multiple sclerosis, Parkinson's disease, myasthenia gravis, and a history of hydrocephalus, nine had a diagnosis of diabetes mellitus, nine had spinal disk herniations or other arthritic abnormalities of the spine, ten had a diagnosed neoplasm, and 11 had other known diseases unrelated to LB. Two patients suffered from more than one disease at the same time.

One hundred and ninety four patients without any symptoms or with symptoms which did not meet inclusion criteria were also excluded. Finally, 423 subjects with the following symptoms were included in the evaluation: joint pain (divided into three minor categories: pain in one large joint, pain in several joints, and pain in only small joints, predominantly the wrists and fingers), joint swelling, muscle pain or muscle cramp, back pain, fatigue, forgetfulness, sensory disorder, headache, visual disorder, and vertigo. These symptoms were considered to be possible consequences of LB. Each patient who reported at least one of these symptoms was tested for antibodies and included in the study. If additional symptoms were present, they were classified as 'others'. This category was not used for statistical analysis. Individuals who only had symptoms in this category were not included in the study population, as mentioned above.

If patients were retested during the study period, only the first visit was considered.

On the basis of the ELISA results, each of the 423 patients was assigned to one of three seropositive groups (IgG positive/IgM positive, IgG positive/IgM negative, and IgG negative/IgM positive) or to the seronegative group. The single-tier criterion was adopted deliberately for assignment in order to assess a 'worst-case scenario'. The immunoblot was performed routinely for all positive ELISA results, and it confirmed approximately 90% of all positive IgG ELISA results and approximately 78% of all positive IgM ELISA results. These proportions did not change between the individual groups. Therefore, we could expect that applying a two-tier criterion would not influence the results.

Descriptive statistics were calculated for baseline parameters for the total patient sample. Continuous variables were described as median and range, and categorical variables as absolute values and percentages.

Single chi-square tests of independence were performed to compare the rates of the non-specific symptoms among the four serological categories. Ninety-five per cent profile CIs were calculated for the individual rates, and are shown in Fig. 2. The multiple analysis of the non-specific symptoms was based on logistic regression models with history of EM and history of tick bite. In addition, we adjusted for age as a confounder. The estimated ORs were tested for significance with z-tests.

p-Values of $\leq$0.05 were considered to be statistically significant. All calculations were performed with R 3.0.2.

Results

In total, 138 (33%) males and 285 (67%) females were included. The median age was 53 years (range, 7–89 years). The demographic details for each antibody category, including history of EM and tick bite, are shown in Table 1.

Three hundred and twenty-four (77%) patients could specify the duration of their symptoms. The median duration of symptoms was 40 weeks, ranging from 1 week to 30 years. In 211 (65%) patients, the duration of symptoms was <1 year.

Table 2 shows the frequencies of non-specific symptoms for each serological category. The results of the univariate logistic regressions are shown in Fig. 2. The null hypothesis of independence could not be rejected for any of the non-specific symptoms. All p-values of the chi-squared tests were above the significance level of 0.05. The percentage of non-specific symptoms did not significantly differ between the seropositive groups and the seronegative controls. Despite the large sample size, the CIs showed a widely overlap, which is evidence that the non-specific symptoms were unrelated to the serological status.

Previous EM was reported by 104 (24%) patients, namely 30 (29%) men and 74 (71%) women. Two hundred and twenty-three (53%) patients reported previous tick bite, namely 77 (35%) males and 146 (65%) females. Comparisons of the frequency of non-specific symptoms between patients with and without a history of EM and tick bite are shown in Table 3. Multivariate analysis of history of EM showed no significant effect on any of the non-specific symptoms. History of tick bite was a weakly significant predictor for joint pain and for joint swelling ($p< 0.05$).

The exact time interval between the EM and the onset of symptoms was known for 64 (15%) patients. Seven patients reported EM twice. In these cases, the time point of the first EM was used. The median time interval was 37 weeks, ranging from 0 weeks to 25 years. In 15 (23%) patients, the non-specific symptoms started simultaneously with the appearance of the rash, 19 (30%) patients reported the onset of symptoms within 1 year after EM, and in the remaining 30 (47%) patients the non-specific symptoms began after 1 year.

Discussion

Serological testing for antibodies against *B. burgdorferi* sensu lato is a cornerstone of the laboratory diagnosis of LB [7]. The highest sensitivity is achieved in the late stage of the disease. However, antibodies can be detected for years in healthy individuals [8,9], even after an asymptomatic infection. The widespread use of serological testing in patients who do not meet the clinical criteria for LB [7] can lead to incorrect diagnosis of the disease, especially if no other reasons for the complaints can be found [10–12]. Many of the patients included in this study had visited different specialists and undergone a

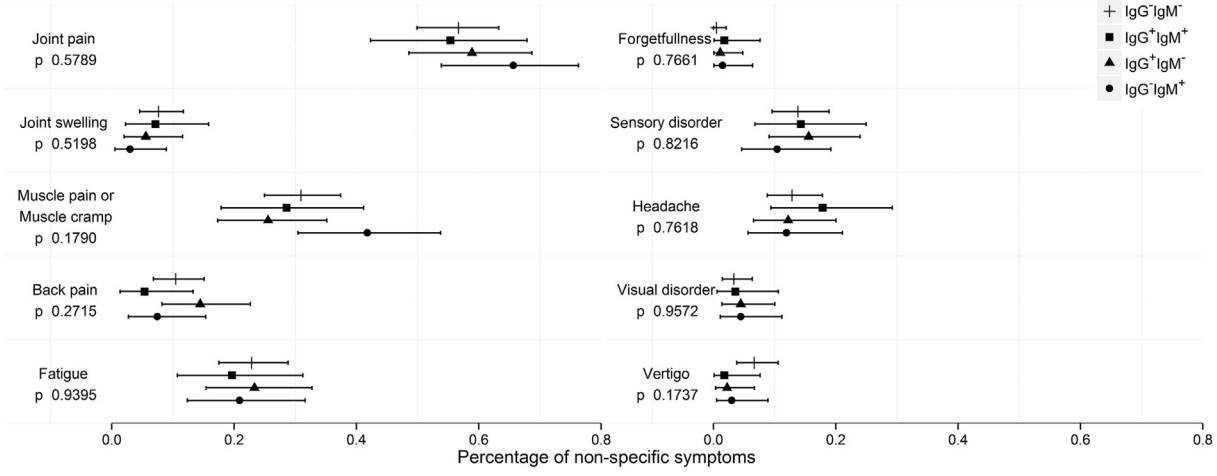

FIG. 2. Impact of antibody status on non-specific symptoms; 95% CIs of the percentages.

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TABLE 1. Demographic data of the study population, including history of erythema migrans (EM) and tick bite

	Sex (absolute frequency (%))		Total (absolute frequency (%))	Age (years)			History of EM (absolute frequency (%))	History of tick bite (absolute frequency (%))
	Male	Female		Median	Minimum	Maximum		
IgG negative IgM negative	63 (30)	147 (70)	210 (100)	48	7	86	36 (17)	102 (49)
IgG positive IgM positive	21 (38)	34 (62)	55 (100)	56	9	76	21 (38)	34 (61)
IgG positive IgM negative	35 (39)	55 (61)	90 (100)	54	23	89	18 (20)	54 (60)
IgG negative IgM positive	19 (28)	49 (72)	68 (100)	53	8	77	29 (43)	33 (49)
Total	138 (33)	285 (67)	423 (100)	—	—	—	104 (24)	223 (53)

variety of investigations in order to find the reasons for their complaints.

In the present analysis, the possible influence of previous infection with *B. burgdorferi* sensu lato on non-specific complaints was investigated. First, the outcomes of seropositive and seronegative subjects were compared. Seroprevalence can be considered as a reliable surrogate parameter for previous infection with the pathogen. In the second step, patients who reported physician-diagnosed EM were compared with patients without a history of EM. A possible influence of infection with *B. burgdorferi* sensu lato on the development of non-specific complaints is described by the so-called 'post-Lyme disease syndrome' (PLDS) [13–15], which includes fatigue and musculoskeletal pain, symptoms that were assessed in the present study. The syndrome is valid only for patients with a documented history of early or late LB after treatment according to recommendations. Patients with a history of EM in the present study correspond, to some extent, with the definition of PLDS. All participants were asked about previous EM that had been diagnosed by a physician and treated accordingly [1].

The evidence for the aetiology of PLDS is not well established. Similar symptoms occur in the general population without a history of LB, which is the major limitation in the investigation of its possible causality [11,12]. The infectious cause of the complaints could not be assessed; identification of a pathogen in patients with non-specific symptoms after antibiotic treatment for LB was not possible. Animal models have shown that antibiotic treatment is highly efficient in clearing the pathogen from the tissue [16]. The post-infectious effect on the immune response without persistence of the pathogen is a current topic of discussion and focus of research [17,18]. Finally, antibiotic treatment was not found to be beneficial for those patients in controlled trials [6,19]. On the other hand, evaluation of the antibody profile in PLDS patients from the USA showed different reactivities to several specific proteins of *B. burgdorferi* sensu lato than in a control population [15]. This finding strengthens the role of the immunological response in this respect.

Different genospecies of *B. burgdorferi* sensu lato cause different clinical manifestations in Europe, whereas, in North America, *B. burgdorferi* sensu stricto is the only agent of LB. Even if a history of LB could be associated with the development of some non-specific symptoms in patients in the USA, it would not necessarily apply to Europe. The majority of studies

TABLE 2. Non-specific symptoms; comparison between antibody categories (absolute frequency (%))

	Joint pain												
	One large joint	Several joints	Small joints	Joint swelling	Muscle pain/muscle cramp	Back pain	Fatigue	Forgetfulness	Sensory disorder	Headache	Visual disorder	Vertigo	Other
IgG negative IgM negative *n* = 210	17 (8)	92 (44)	10 (5)	16 (8)	65 (31)	22 (9)	48 (23)	1 (0)	29 (14)	27 (13)	7 (3)	14 (7)	53 (25)
IgG positive IgM positive *n* = 55	5 (9)	24 (43)	2 (4)	4 (7)	16 (29)	3 (5)	11 (20)	1 (2)	8 (14)	10 (18)	2 (4)	1 (2)	10 (18)
IgG positive IgM negative *n* = 90	4 (4)	43 (48)	6 (7)	5 (6)	23 (26)	13 (14)	21 (23)	1 (1)	14 (16)	11 (12)	4 (4)	2 (2)	9 (10)
IgG negative IgM positive *n* = 68	9 (6)	33 (49)	5 (7)	2 (3)	28 (42)	5 (7)	14 (21)	1 (1)	7 (10)	8 (12)	3 (4)	2 (3)	13 (19)
Total *n* = 423	35 (8)	192 (45)	19 (4)	27 (6)	132 (31)	43 (10)	94 (22)	4 (1)	58 (14)	56 (13)	16 (4)	19 (4)	85 (20)

TABLE 3. Non-specific symptoms; comparison between patients with and without a history of erythema migrans (EM), and with and without a history of tick bite (absolute frequency (%))

	Joint pain												
	One large joint	Several joints	Small joints	Joint swelling	Muscle pain/muscle cramp	Back pain	Fatigue	Forgetfulness	Sensory disorder	Headache	Visual disorder	Vertigo	Other
History of EM	11 (11)	45 (43)	6 (6)	4 (4)	41 (39)	10 (10)	19 (18)	1 (1)	11 (11)	11 (11)	3 (3)	1 (1)	14 (13)
No history of EM	21 (7)	147 (46)	17 (5)	23 (7)	91 (29)	33 (10)	75 (24)	3 (1)	47 (15)	45 (14)	13 (4)	18 (6)	71 (22)
History of tick bite	14[a] (6)	112[a] (50)	14[a] (6)	9[a] (4)	70 (31)	25 (11)	53 (24)	3 (1)	24 (11)	27 (12)	9 (4)	13 (6)	47 (21)
No history tick bite	18[a] (9)	80[a] (40)	9[a] (4)	18[a] (9)	62 (31)	18 (9)	41 (20)	1 (0)	34 (17)	29 (14)	7 (4)	6 (3)	38 (19)

[a]$p < 0.05$ (calculated for joint pain as a single category).

dealing with non-specific symptoms among European patients with Lyme disease were treatment trials focusing on outcomes after different antibiotic regimens [20–23]. In a European prospective clinical trial, it was shown that the frequency of non-specific symptoms in patients treated for EM did not exceed the frequency of such symptoms in a control group without a clinical history of LB [24]. Greater severity of symptoms was found in patients with a history of EM than in controls; however, greater sensitivity to the symptoms in this group might explain this finding. Similar conclusions regarding EM and non-specific symptoms can be drawn from present evaluation. Note that the role of disseminated LB in this respect has not been analysed.

Surprisingly, a history of tick bite showed a positive correlation with joint pain and joint swelling. To explain this dependence, one can argue that patients who have symptoms of unknown origin may be more likely to associate them with a previous tick bite and to consider them to be a potential consequence of LB. For this reason, they may pay more attention to previous tick bites than individuals without symptoms. On the other hand, there might be another, unknown, trigger for the development of the symptoms caused by tick bites. The present analysis clearly shows that an aetiological role of borrelial infection is highly improbable in this respect.

The exclusion of asymptomatic patients (Fig. 1) in this study deserves some explanation. The majority of patients without symptoms have already shown positive serological test results in another laboratory. Therefore, the frequency of seropositive subjects in the asymptomatic group could be overestimated in relation to the general population. To overcome this problem, only patients with certain, well-defined symptoms were included in the evaluation.

Patients with some known underlying diseases were excluded from the evaluation in order to rule out a possible overlap between the disease and the potential influence of LB on the symptoms. One can argue that this step was arbitrary, as some chronic diseases do not cause all of the symptoms investigated in the study. For instance, diabetes mellitus can cause polyneuropathy resulting in sensory disorder, but it is rather less probable that it can cause joint pain. However, the total number of such subjects was low as compared with the total sample size. For instance, there were five patients with diabetes and joint pain. Among patients with different neurological diseases, five reported muscle pain and two reported joint pain. The inclusion of these patients in the analysis did not considerably change the results of the study.

Five patients with a history of proven LNB were not included in the evaluation. Clinical follow-up of these patients should be discussed. All were treated with ceftriaxone. The first patient received a diagnosis of LNB twice, 9 years and 2 years previously. Several painful complaints were reported afterwards; however, the symptoms could also be explained by vertebrostenosis, diagnosed with magnetic resonance imaging. Of the remaining four patients, two reported lumboischialgia 1 year and 10 years after the diagnosis of LNB, respectively. Another patient, a 12-year-old child, reported ankle pain and tiredness 7 months after the diagnosis. Because of the small number of patients and the lack of full diagnostic work-up, it is difficult to confirm a possible influence of previous LNB on these symptoms. The last patient was symptom-free 3 months after treatment. In three patients who presented with Bannwarth syndrome, LNB was confirmed with lumbar puncture later on. All patients presented for follow-up visits, and all of them were symptom-free after 4 months, 1 year, and 2 years, respectively.

Some limitations of the study should be mentioned. Although patients with known underlying diseases were excluded from the evaluation, some of the participants might have had other unknown diseases [25,26]. The majority of patients had been seen by other specialists, and no definitive diagnosis had been made; thus, a diagnosis of LB was considered to be possible. It should be noted that seropositivity and anamnestic EM were used as surrogate parameters for the previous infection. However, patients with an early stage of the disease or who have received early treatment may not develop specific antibodies. Furthermore, not all patients recall EM or tick bites.

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Finally, patients with disseminated LB regardless of antibody status could not be included in the present study design.

Conclusions

The results show that testing patients with non-specific symptoms for antibodies against *B. burgdorferi* sensu lato in the everyday clinical setting does not provide any useful information about their aetiology. An aetiological role of *B. burgdorferi* sensu lato in the development of self-reported complaints in seropositive patients and in patients with history of EM is rather unlikely.

Transparency declaration

The authors declare no conflicts of interest.

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