Clinical evaluation of guidelines and two-test approach for Lyme disease

A. A. M. Blaauw, A. M. van Loon, J. F. P. Schellekens and J. W. J. Bijlsma

Department of Rheumatology and Clinical Immunology and 1Department of Virology, University Medical Centre, PO Box 85500, 3508 GA Utrecht and 2National Institute of Public Health and the Environment, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, 3720 BA Bilthoven, The Netherlands

Abstract

Objective. The diagnosis of Lyme disease should be based on objective clinical signs and symptoms. In a clinical study, we have evaluated whether the recommended two-step approach for serodiagnosis of Lyme disease is useful in daily clinical practice and can influence clinical decision making.

Methods. The signs and symptoms of patients with ongoing musculoskeletal complaints, assumed by their referring physician or themselves to be attributable to active or chronic Lyme disease, and of patients diagnosed as having Lyme disease, were evaluated. On the basis of clinical evaluation only, patients were classified into three groups: previous Lyme disease, active Lyme disease and no Lyme disease. Antibodies to *Borrelia burgdorferi* were determined by means of an enzyme-linked immunosorbent assay (ELISA), followed, when positive, by immunoblotting.

Results. One hundred and three patients (41 males and 62 females, mean age 48.7 yr) participated in the study. Of the 49 patients classified as previous Lyme disease, 25 (51%) had antibodies to *B. burgdorferi*. All 10 patients with active Lyme disease had positive antibodies and 12 of the 44 patients (27%) classified as no Lyme disease had positive antibodies. No statistically significant differences were found between the percentage of positive immunoblots from patients with previous Lyme disease (72%) and patients with active Lyme disease (100%). In the group of no Lyme disease, five out of 12 patients had a negative immunoblot. Concerning serological testing, immunoblotting could have added additional information. However, immunoblotting did not influence clinical decision making in this group of patients.

Conclusion. Immunoblotting did not influence clinical decision making for the 47 patients with antibodies to *B. burgdorferi* in this study.

Key words: Lyme disease, Lyme serology, Immunoblot.

The clinical manifestations of Lyme disease have been well documented since its first description as a distinct entity in 1977 [1, 2]. In Europe, at least three distinct species of *Borrelia burgdorferi* sensu lato can cause the clinical syndrome of Lyme disease: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Clinical manifestations correlate with the genospecies causing the infection. The clinical diagnosis of Lyme disease should be based on objective clinical signs and symptoms. Too often, the diagnosis is made in patients with non-specific and atypical features, such as headache, fatigue, arthralgia and myalgia. This has led to ‘overdiagnosis’ of Lyme disease [3–5].

In Europe, no clinical diagnostic criteria for Lyme disease have been developed so far. Criteria developed in the USA by the Centers for Disease Control and Prevention (CDC) for surveillance purposes are often used as clinical criteria in the USA and in Europe. Since it is rather difficult to culture *B. burgdorferi* from specimens other than erythema migrans lesions, especially in daily clinical practice, the presence of antibodies to *B. burgdorferi* may confirm the clinical diagnosis. Although serological testing for Lyme disease can be performed with a high degree of sensitivity, false-negative and false-positive results continue to be an important problem [3]. Patients with Lyme disease usually remain seropositive for IgG antibodies to *B. burgdorferi* for years, in some cases even permanently.
In these patients, a positive test can lead to ‘overdiagnosis’ and probably ‘overtreatment’. False-positive results have been reported for patients with rheumatoid arthritis, systemic lupus erythematosus, infectious mononucleosis, syphilis and other spirochaetal diseases [7]. In Europe, asymptomatic seropositivity determined by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assays (IFA) has also been demonstrated in up to 20% of the normal population as well as people at risk, such as orienteers, hunters and foresters [8–11]. As a consequence, in the absence of typical clinical signs and symptoms, diagnostic testing for antibodies to B. burgdorferi is of limited value [10–14].

Although no consistent evidence suggests that Western blotting adds additional information to an accurate clinical history or to the results of a positive or indeterminate ELISA or indirect IFA if used in all patients, Dresser et al. and the CDC suggest that a two-step testing strategy should be used [13, 15, 16]. This two-step strategy is also adopted in Europe. Western blotting can detect and discriminate among antibodies to multiple antigens of B. burgdorferi. However, it remains unclear whether Western blotting can discriminate between true-positive test results and false-positive test results, and between active or previous infection with B. burgdorferi [17, 18]. Therefore, the clinical implications of this recommended two-step approach for the serodiagnosis of Lyme disease remain unclear and even questionable.

In this clinical study, we studied a patient population in which questions such as a true- or false-positive test result and active or previous infection are important. Clinical criteria were used as a gold standard, and ELISA and immunoblot test results were used and interpreted as in daily clinical practice. We have tried to assess whether immunoblotting should be performed in all patients with a positive ELISA and whether the suggested two-step approach can influence clinical decision making.

**Patients and methods**

**Patients**

Consecutive patients of the out-patient clinic of the Department of Rheumatology and Clinical Immunology of the University Medical Centre Utrecht, The Netherlands, were evaluated. Patients with a classic presentation of Lyme disease such as erythema migrans are, in general, diagnosed and treated by their general practitioners. Because of our special interest in clinical Lyme disease, the department serves as a secondary or tertiary referral centre for patients with presumable Lyme disease or for patients with persisting complaints thought to be chronic Lyme disease.

Patients eligible for this study were (i) patients with persisting musculoskeletal complaints assumed by their referring physicians to be attributable to active or chronic Lyme disease, (ii) patients who believed that they had active or chronic Lyme disease and (iii) patients with musculoskeletal complaints diagnosed as Lyme disease by our staff members.

All patients were seen by one of us (AAMB) who recorded a medical history and performed the physical examination. Questions were asked about past or recent tick exposure and the characteristic manifestations of Lyme disease. Clinical records and former medical correspondence were studied.

In the daily routine of our department, it is not possible to culture B. burgdorferi from clinical specimens. Genome amplification methods and urine antigen analysis are only used in experimental studies. It was therefore decided that clinical criteria were to be used as the ‘gold standard’. Patients were classified according to clinical criteria independent of the serological test results of (ELISA) antibodies to B. burgdorferi both at the time of (presumed) diagnosis and during the study. Because of the special referral pattern of patients seen in our department, we expected that if these patients had antibodies to B. burgdorferi, these would be IgG antibodies. IgM antibodies were not determined. If patients had (ELISA) IgG antibodies to B. burgdorferi, an IgG immunoblot was performed.

No exact figures about the incidence and prevalence of Lyme disease in The Netherlands exist. The incidence is assumed to be low and is estimated to vary from 0.01% in the general population to 2–9% in populations at risk and patients with monoarticular arthritis [8, 10, 19].

**Clinical criteria**

On the basis of objective clinical signs and symptoms only, the patients were divided into three groups: previous Lyme disease, active Lyme disease and no Lyme disease.

The clinical criteria for Lyme disease according to literature references were defined as follows [6]: erythema migrans is defined as a red macula or papule that expanded over a period of days to weeks to form a large annular lesion, at least 5 cm in size, often with partial central clearing [1, 20, 21]. The skin lesion should have been confirmed by a physician or recognized by the patient from pictures of erythema migrans [10]. Early neurological involvement includes lymphocytic meningitis, cranial neuritis and radiculopathy, accompanied by pleocytosis of the cerebrospinal fluid [22–24]. Chronic neuroborreliosis includes encephalopathy with memory impairment, polyneuropathy with radicular pain or distal paraesthesiae and leucoencephalitis with spastic paraparesis [25]. Lyme arthritis is defined as recurrent, brief attacks of objective joint swelling in one or a few joints, especially the knees, possibly followed by chronic synovitis [26]. Lyme carditis is characterized by fluctuating degrees of atioventricular nodal block that resolved in days to weeks, possibly associated with myocarditis [27]. Uveitis is defined as intermediate uveitis with characteristic spider web vitritis confirmed by an ophthalmologist [28]. Acrodermatitis chronica atrophicans (ACA) is the characteristic bluish-red discolouration, often with a doughy infiltration and
progressing to atrophy or sclerodermic changes [21]. Borrelial lymphocytoma is the typical bluish-red tumour-like infiltrate [21]. For all patients with neurological, cardiac, eye or joint abnormalities, all other possible causes of the complaints had to be excluded. Skin lesions that developed immediately after a tick bite were not considered to be erythema migrans. Aspecific symptoms such as fatigue and arthralgia, fever, headache and paraesthesiae as a single symptom, palpitations, bundle branch block on electrocardiography, myocarditis and symmetrical polyarthritus were not accepted as clinical criteria for Lyme disease unless accompanied by objective caracteristic Lyme manifestations mentioned above. A tick bite alone was also not accepted as a clinical criterion.

A clinical diagnosis of previous Lyme disease was made if at least one of the clinical criteria was present in the past and no objective manifestations of Lyme disease were present at the time of the study. Active Lyme disease was diagnosed when at least one of the clinical criteria was present at the time of the study. When no clinical criteria were present or had been present in the past, patients were assumed to have no Lyme disease.

Enzyme-linked immunosorbent assay

In daily clinical practice, our hospital uses a commercial ELISA for the detection of antibodies to B. burgdorferi (Dako A/S, Glostrup, Denmark) [29, 30]. In all cases, the IgG antibody response to B. burgdorferi was determined by this commercial ELISA. If a positive test result was obtained, the Treponema pallidum haemagglutination assay was performed to exclude false positivity due to antibodies to T. pallidum.

Patients were tested at the time they were seen in our outpatient clinic. Because of our special patient population, it is possible that patients are seen not only if they have active symptoms of Lyme disease but also years after a (presumed) diagnosis of Lyme disease. No follow-up samples were determined.

Western blot analysis

For all patients with IgG antibodies to B. burgdorferi according to the ELISA assay, IgG immunoblot analysis was performed as described in the literature [16, 30]. The IgG immunoblot was considered positive if at least five of the following eight bands were present: 17 kDa, 22 kDa, 31 kDa, 34 kDa, 39 kDa, 41 kDa, 58–74 kDa and 92 kDa, or if four bands were positive including 17 kDa or 22 kDa or 39 kDa or 92 kDa. One or more bands in the 58–74 kDa region were considered as one band [31, 32]. IgG immunoblot was considered negative if three bands were positive excluding 17 kDa, 22 kDa, 39 kDa or 92 kDa, and if less than three bands were found to be positive. IgG immunoblot was considered equivocal if four positive bands (31 kDa, 34 kDa, 41 kDa and 58–74 kDa) were found, or only three bands including 17 kDa or 22 kDa or 39 kDa or 92 kDa. Using the above criteria, the sensitivity of IgG immunoblot is 45% in early Lyme disease and 95% in disseminated or late Lyme disease; the specificity of IgG immunoblot is 98% (unpublished data, personal communication). The control population in which IgG immunoblot was tested consisted of healthy donors, pregnant women and potentially cross-reactive sera of patients with syphilis, lepromatosis or autoimmune diseases. The clinical diagnosis defined by a clinical expert was considered as the ‘gold standard’.

In our hospital, the clinician receives the results of immunoblotting interpreted by the laboratory as either positive, negative or equivocal. The clinician is unaware of which bands of the immunoblot are positive or negative. No follow-up samples were determined.

Statistics

For categorical data, Fisher’s exact test was used to test for differences between groups. A P value of < 0.05 was considered statistically significant.

Results

In a 4 yr period (April 1994–April 1998), 105 patients who met either one of the three eligibility criteria were seen in our department. In two patients, the ELISA for antibodies to B. burgdorferi was not performed at study entry. Of the remaining 103 patients, 41 (40%) were male and 62 (60%) were female. The mean age of the patients was 48.7 yr (range 6–82 yr).

The 103 patients were grouped according to the clinical criteria: 49 patients were classified as previous Lyme disease (48%), 10 patients as active Lyme disease (10%) and 44 as no Lyme disease (42%) (Table 1). Forty-seven of the 103 patients (46%) had IgG antibodies to B. burgdorferi according to the ELISA at study entry; 25 patients with previous Lyme disease (51%), all 10 patients with active Lyme disease (100%) and 12 patients (27%) with no Lyme disease (Table 1). Results are summarized in Table 2.

Patients classified as previous Lyme disease

No differences for gender or mean age were found for the 49 patients classified as previous Lyme disease who did or did not have antibodies to B. burgdorferi.

Of the 25 patients with antibodies to B. burgdorferi, 15 had had early symptoms of Lyme disease defined as signs or symptoms within < 1 yr after possible exposure. Fourteen of these patients had erythema migrans and one patient had neuroborreliosis. Ten patients had late symptoms defined as signs and symptoms > 1 yr after possible exposure. Two of these 10 patients had ACA, one had uveitis and seven had mono- or oligoarthritis of the knees (six patients) and elbow (one patient).

Of the 24 patients without antibodies to B. burgdorferi, 19 had erythema migrans, one had erythema migrans and neuroborreliosis, and three had neuroborreliosis. One patient had late symptoms: arthritis of the knee.

Patients with antibodies to B. burgdorferi were seen on average 3.9 yr after the diagnosis of Lyme disease was made elsewhere, patients without antibodies on average after 4.6 yr. At study entry, none of these 49
patients had objective signs of Lyme disease. In 51% of these patients, antibodies to \textit{B. burgdorferi} could be detected after all these years.

\textbf{Patients with active Lyme disease}

Ten patients were classified as having active Lyme disease based on clinical symptoms: one patient had neuroborreliosis with radiculopathy and lymphocytic meningitis, three patients had ACA, six patients had episodes of recurrent arthritis of the knee, one of them after erythema migrans. All patients received appropriate antibiotic treatment. All patients with active Lyme disease had IgG antibodies to \textit{B. burgdorferi}. All patients contracted Lyme disease in Europe, except for the patient with neuroborreliosis who developed signs and symptoms after a trip to the east coast of the USA.

\textbf{Patients with no Lyme disease}

According to our clinical criteria, 44 patients did not have objective signs of Lyme disease at study entry. Based on clinical evaluation and chart and correspondence review, they did not have objective signs or symptoms either, at the time a presumable diagnosis of Lyme disease was made elsewhere. Thirty-two patients did not have IgG antibodies to \textit{B. burgdorferi} at the time of study entry. However, 12 of these 44 patients (27%) did have IgG antibodies to \textit{B. burgdorferi}.

\textbf{Immunoblot}

An IgG immunoblot was performed in all 47 patients with IgG antibodies to \textit{B. burgdorferi}. Of the 25 patients classified as previous Lyme disease, 18 had a positive immunoblot (72%) and seven had a negative immunoblot. The seven patients with a negative immunoblot did have objective signs and symptoms of Lyme disease in the past. ELISA test results of these patients cannot be regarded as false positive in these seven patients. Because of the time interval between the diagnosis of Lyme disease and the time of the study, a waning humoral immunity with a ‘rest-response’ to the 41 kDa protein is possible. All 10 patients with active Lyme disease had a positive immunoblot (100%). No statistically significant difference was found between the percentage of positive immunoblots from patients with previous Lyme disease and those with active Lyme disease. Based on clinical criteria, however, it was possible to differentiate between previous and active Lyme disease. Therefore, the use of the immunoblot assay in these two patient groups did not add any additional information to the results of the ELISA, and did not influence clinical decision making.

In the group of no Lyme disease, seven of 12 patients (58%) had a positive immunoblot and five patients (42%) had a negative immunoblot. Concerning serological testing, immunoblotting could have added additional information for five patients in this group of no Lyme disease: ELISA results were probably false positive. However, based on the clinical criteria (independent of any serological results), these patients did not have signs of Lyme disease. Therefore, immunoblotting did not influence clinical decision making for this group of patients.

Seven patients classified as no Lyme disease had positive ELISA IgG antibodies to \textit{B. burgdorferi} and a positive IgG immunoblot. Clinical characteristics of these patients are discussed briefly. A 37-yr-old male had systemic sclerosis and arthralgia. Because of antibodies to \textit{B. burgdorferi}, he was treated with several courses of ceftriaxone i.v. without any clinical effect. A 40-yr-old male had symmetrical, rheumatoid factor-
negative polyarthritis. Treatment with doxycycline and ceftriaxone had no effect. He was started on sulphasalazine as a second-line anti-rheumatic drug with excellent response. A 72-yr-old male had symmetrical, rheumatoid factor-positive, erosive polyarthritis classified as typical rheumatoid arthritis. A 56-yr-old male had eczema and arthralgia. He never had objective signs of arthritis. A 17-yr-old male was referred because of a tick bite in the past and antibodies to B. burgdorferi. He did not have any complaints or objective signs or symptoms. A 52-yr-old female had arthralgia without objective arthritis. A 23-yr-old female had arthralgia and painful knees without signs of arthritis, classified as chondromalacia patellae.

The positive results of ELISA and immunoblot in these seven patients have to be considered as true, but asymptomatic, positive. On clinical criteria, we do not feel that these patients have or had Lyme disease.

Discussion

In this study, we evaluated recommendations for the use of a two-test approach for Lyme disease in a clinical situation. We especially assessed whether clinical decision making was influenced by the suggested two-test approach: a positive or undetermined ELISA should be followed by Western blot. The results indicate that immunoblotting does not reliably discriminate between previous infection and active infection with B. burgdorferi. Clinical decision making was not influenced by the results.

Twelve out of 44 patients classified as no Lyme disease at study entry had IgG antibodies to B. burgdorferi. This percentage is in contrast with the percentage of antibodies to B. burgdorferi found in the Dutch population and patients at risk [8, 10, 11]. This high percentage is probably due to referral bias. It is likely that patients with antibodies are referred, as patients without antibodies are not. Seven of these 12 patients did have a positive immunoblot. Based on their medical history and the clinical picture, these patients had not had Lyme disease. In this group of patients classified as no Lyme disease, the use of immunoblotting could have added additional information for the five patients whose immunoblot was negative. However, based on clinical criteria, these patients did not have Lyme disease at study entry or in the past. Thus, in this group of patients with no Lyme disease, the immunoblot did not influence clinical decision making either. Our study supports and strengthens the suggestion that the diagnosis of Lyme disease should be made primarily on clinical signs and symptoms [33]. Neither the results of serological testing nor the results of immunoblotting can be interpreted adequately without knowledge of clinical manifestations. Our results confirm the findings of Gern et al. [34] who stated that immunoblotting is of little help in diagnosing Lyme disease in populations at risk as well as in endemic areas where seropositivity for B. burgdorferi is common.

A few points should be stressed. First, there is no gold standard for the clinical and laboratory diagnosis of Lyme disease. No generally accepted criteria have been developed, although ‘practice parameters’ and guidelines for the diagnosis of patients with Lyme borreliosis of the nervous system and Lyme arthritis have been proposed and developed [22, 35]. Overdiagnosis as well as underdiagnosis of erythema migrans, considered as the hallmark of the disease, have been described [36, 37]. For this particular study, we used diagnostic criteria based on the literature as well as our own experience with Lyme disease patients [2, 3, 5, 12, 13, 21, 22]. We grouped our patients on the basis of these clinical criteria independently of the serological results, in an attempt to identify the possible surplus value of serology and immunoblotting. Patients could easily be assigned to one of the three groups previous Lyme disease, active Lyme disease and no Lyme disease.

Second, our study was performed in a referral hospital with a population of patients which is not representative of the total population of patients with disease due to infection with B. burgdorferi. However, these patients are a reflection of the problems which are encountered in the daily management of patients with (presumed) Lyme disease: active infection, past infection with persistent antibodies to B. burgdorferi, false-positive or asymptomatic IgG antibodies to B. burgdorferi. We have not provided any data on sensitivity, specificity, and the positive or negative predictive values of the ELISA and immunoblotting, because these data would only be applicable to a similar population and cannot be generalized for other populations. We used a standard commercial ELISA for this study as will be used in daily clinical practice by most physicians. We are aware that more sensitive assays will be available in the future. However, the possible surplus value of all these assays should be evaluated against clinical criteria.

Third, based on clinical criteria, we classified 44 patients as no Lyme disease. We used only objective signs and symptoms in our criteria. None of these 44 patients ever had any of these symptoms. This would have made their pre-test likelihood of Lyme disease <20%. These patients should not have been tested for the presence of antibodies to B. burgdorferi in the first place because a positive test is more likely to be clinically false positive than true positive [10, 13]. We assumed that it is likely that referring physicians use a negative result of ELISA for antibodies to B. burgdorferi to rule out the presence of Lyme disease and refer patients with a positive test result to our out-patient clinic.

Fourth, we studied a relatively small patient population with either positive or negative ELISA results. Larger study populations are needed for definite conclusions and for patients with possible indeterminate ELISA results. However, it can be expected that if clinicians determine the pre-test probability of Lyme disease in the diagnostic evaluation of a patient for Lyme disease, based on findings of a thorough clinical examination and knowledge of the incidence of Lyme disease in the population represented by the patient, testing for antibodies to B. burgdorferi can be avoided in patients with non-specific symptoms [13].
In view of the results of this study, we cannot recommend that, in a clinical situation, a positive ELISA for antibodies to *B. burgdorferi* should always be followed by immunoblotting. Immunoblotting did not influence clinical decision making for 47 patients with positive ELISA antibodies to *B. burgdorferi*. Results of serological testing and immunoblot analysis should not be interpreted without sufficient knowledge of the clinical picture of the patients tested.

**References**


