Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe

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Abstract

Lyme borreliosis, caused by spirochaetes of the *Borrelia burgdorferi* genospecies complex, is the most commonly reported tick-borne infection in Europe and North America. The non-specific nature of many of its clinical manifestations presents a diagnostic challenge and concise case definitions are essential for its satisfactory management. Lyme borreliosis is very similar in Europe and North America but the greater variety of genospecies in Europe leads to some important differences in clinical presentation. These new case definitions for European Lyme borreliosis emphasise recognition of clinical manifestations supported by relevant laboratory criteria and may be used in a clinical setting and also for epidemiological investigations.

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Introduction

Lyme borreliosis (LB), caused by spirochaetes of the *Borrelia burgdorferi* sensu lato genospecies complex, is the most commonly reported tick-borne infection in Europe and North America [1] The disease was referred to as Lyme arthritis following investigation in the town of Old Lyme, Connecticut, USA, in the mid 1970's into a geographical cluster of suspected juvenile rheumatoid arthritis cases that were shown to be associated with tick bites [2]. Further studies led to isolation of an extracellular spirochaete from the deer tick, *Ixodes scapularis (dammini)* [3], subsequently named *Borrelia burgdorferi* [4]. Following recognition that this organism can cause a multi-system disorder affecting a range of tissues including joints, skin, heart, nervous system, and to a lesser extent some other organs [5], the term Lyme arthritis was adopted for the arthritic features of the disease and Lyme disease for the whole spectrum. Many features of the disease had been known much earlier in Europe under a variety of names including erythema (chronicum) migrans, lymphadenosis benigna cutis, acrodermatitis chronica atrophicans, meningopolyneuritis (also known as Garin-Bujadoux-Bannwarth syndrome) [6]. In Europe the disease is generally referred to as Lyme borreliosis (LB).

LB in Europe and in North America are very similar in their main clinical features, but differ in some aspects, evidently due to the greater variety of genospecies that cause disease in Europe (*B. afzelii, B. garinii, B. burgdorferi* sensu stricto, and occasionally other species such as *B. spielmanii*), whereas in the US *B. burgdorferi* sensu stricto is the only pathogenic genospecies [7,8]. Acrodermatitis chronica

atrophicans and borrelial lymphocytoma very rarely occur in the US, but are well-recognised in Europe.

Erythema migrans in Europe, caused by *B. afzelii*, expands more slowly and central clearing is more frequent than in the US where the same manifestation is caused by *B. burgdorferi*. However, in American patients systemic symptoms and seroreactivity are more frequent [9]. Lyme neuroborreliosis appears to occur in a higher proportion of patients in Europe, and Lyme arthritis seems to be a more frequent manifestation in the US. However, data on relative frequency is limited and bias in reporting systems in both the US and Europe have made it difficult to reach reliable conclusions [10].

Few countries in Europe have made LB a compulsorily notifiable disease, therefore it is possible to make only approximate estimates of LB incidence. In most countries assessment is mainly conducted through diagnostic laboratories reporting on the available details of patients with positive tests. There are several drawbacks involved in using such systems for the estimation of LB incidence, including under-reporting of erythema migrans, varying patterns of test referrals, varying serodiagnostic criteria and seropositivity linked to past exposure. Within these limitations it is possible to gain useful information from individual countries' systems through year-to-year comparisons of within-country data. It is apparent that LB shows a gradient of increasing incidence from west to east with the highest incidences in central Europe (e.g. Slovenia, 155/100,000) and the lowest in the UK (0.7/100,000) and Ireland (0.6/100,000). The real incidence is most probably higher. A gradient of decreasing incidence from south to north in Scandinavia and north to south in Italy, Spain and Greece is also evident [11].

Public perceptions of the disease in Europe have been distorted by the media and by activist groups, with exaggerated claims of pathogenicity and of difficulties of diagnosis and treatment. While it is true that LB may present diagnostic and treatment problems, many of the misconceptions result from misdiagnosis of LB in patients who have other conditions. This can occur because some clinical presentations of LB are not unique to that infection, and also because inadequately standardized and quality-controlled diagnostic methods are used in some laboratories.

Such problems have necessitated the production of case definitions, primarily for epidemiological purposes in the USA [12] (updated in 2008 [13]) but for clinical management purposes in Europe [14]. Since the publication of the first European case definitions in 1996 the health burden imposed by LB has increased, partly because of increased incidence in some regions [15–19]. An additional factor has been the growth of activist groups promoting nonspecific case definitions and unorthodox, unvalidated diagnostic tests and treatment regimens that have led to misdiagnoses and adverse events. This problem has been much more prominent in the USA, but has increased dramatically in Europe in recent years, causing harm to patients and creating pressure on both physicians and politicians. The first European case definitions [14] produced by the European Union Concerted Action on Lyme Borreliosis (EUCALB), an EU-funded initiative, were formulated after wide consultation across Europe. The updated European case definitions presented here have been produced by clinicians on the EUCALB Advisory Board in order to clarify and improve the diagnosis and management of LB, and are based on evidence from the peer-reviewed international literature and on the broad clinical and laboratory experience of the authors.

The literature search strategy included the references in the first European case definitions [14] and, for more recent observations and findings, of searches in Medline, Scopus and Web of Science for worldwide publications using the following keywords 'Lyme or borreliosis or borrelia or erythema migrans or neuroborreliosis or borrelia lymphocytoma or acrodermatitis atrophicans or Lyme carditis or Lyme arthritis or Lyme encephalopathy or chronic Lyme or post-Lyme'. Some pre-1996 references that made important contributions to the literature on the emergence of LB or that concern significant diagnostic principles or methodologies have also been included.

In order to aid reader evaluation of the literature, we have created a basic categorization system of the publications cited, as follows:

I. Randomized trials involving adequate numbers of subjects for statistical analysis.
II. Trials with adequate numbers for statistical analysis, but without randomization e.g. cohort, case-controlled, multiple time-series studies.
III. Clear findings from uncontrolled studies (e.g. case reports).
IV. Opinions of and descriptive studies by relevant authorities or expert committees (e.g. reviews, guidelines).

These publication categories have been applied to the listed references.

Note that the above categorization is distinct from the system used by the Infectious Disease Society of America for assessing quality of evidence in clinical guidelines [20].

Manifestations of European Lyme Borreliosis

For a diagnosis of LB to be considered, the patient must have been exposed to the risk of tick bite. A history of

documented tick bite is not essential because many tick bites go unnoticed.

Skin manifestations

Erythema migrans. Erythema migrans (EM; previously referred to as erythema chronicum migrans) usually occurs several days to weeks after a tick bite. The lesion starts from a macule or papule and expands over a period of days to weeks to form a red or bluish-red patch, with or without central clearing. The advancing edge is typically distinct and is often intensely coloured but not markedly elevated [14]. Most EMs seen by clinicians are more than five cm in diameter but can still be diagnosed by experienced clinicians when smaller. Multiple EMs may also occur. The EM may be accompanied by fatigue, fever, headache, mild stiff neck, arthralgia and myalgia, but such symptoms are not indicative of LB if they occur in the absence of EM [21,22]. EM may be evident for several months but is self-limiting. However, without appropriate treatment other manifestations may follow as a result of spirochaete dissemination to other tissues. Immunosuppression apparently has no statistically significant effect on the clinical presentation, serology or treatment outcome for EM [23,24]. Erythematous lesions occurring within a few hours after a tick bite represent hypersensitivity reactions and do not qualify as EM. Other differential diagnoses may include insect bite reactions, urticaria, contact eczema, cellulitis, folliculitis, erysipelas, tinea corporis, granuloma annulare, or fixed drug eruption.

The diagnosis of EM is clinical. Serological results in patients with early lesions are frequently negative but positive results do not prove EM because background seropositivity is high in some regions [25,26]. Furthermore, prompt antibiotic treatment of this manifestation may ablate the antibody response [27]. Serology does not contribute significantly to a diagnosis of EM. If an atypical manifestation of EM is suspected clinically, detection of spirochaetes in biopsies from suspected lesions by culture and/or PCR is helpful in proving *B. burgdorferi* s.l. infection.

Borrelial lymphocytoma. Borrelial lymphocytoma (lymphadenosis benigna cutis) is rare. It is a painless bluish-red nodule or plaque, usually found on the ear lobe, ear helix, nipple or scrotum and occurs more frequently in children (especially on the ear) than in adults [14]. In the absence of appropriate treatment the lesion may persist for months and other manifestations of LB may follow [28]. Patients with borrelial lymphocytoma are usually seropositive at the time of presentation. The small proportion who initially have negative results usually seroconvert within a short period [29]. Histology is required where there is diagnostic uncertainty to exclude cutaneous lymphoma or other malignancies. Borrelial lymphocytoma has a typical histological appearance with an intense polyclonal B-lymphocytic infiltrate [30].

Acrodermatitis chronica atrophicans. Acrodermatitis chronica atrophicans (ACA) is almost exclusively seen in adults, predominantly women, though ACA-like lesions in children have been reported occasionally [31]. It is a long-lasting, usually progressive manifestation of LB, characterised by red or bluish-red lesions, usually on the extensor surfaces of the extremities. Initially there is a bluish-red discolouration, often with doughy swelling. Later on skin atrophy becomes more and more prominent. Fibroid nodules may develop over bony prominences and sclerodermic changes may develop in atrophic skin areas [14]. The lesion has a typical histological appearance with telangiectases, a patchy or band-like lymphocytic and plasma cell infiltrate, and a greater or lesser degree of skin atrophy, which, however, is not diagnostic *per se* [32]. Involvement of peripheral nerves is not uncommon, locally at the site of the skin lesion, usually as large-fibre axonal polyneuropathy with predominantly mild sensory symptoms [33,34]. Serum IgG antibodies to *B. burgdorferi* s.l. are present in high concentrations.

The differential diagnosis of ACA depends on the stage of the disease. ACA skin lesions on lower extremities are often falsely interpreted to be a result of vascular insufficiency (e.g. chronic venous insufficiency, superficial thrombophlebitis, hypostatic eczema, arterial obliterative disease), acrocyanosis, livedo reticularis, lymphedema, a consequence of old age ('old skin') or chilblains. Fibrous nodules are often misinterpreted as rheumatoid nodules and sometimes as skin involvement in the course of gout (tophi) or even as erythema nodosum. It is not unusual for patients with ACA to consult their doctors because of difficulties with footwear associated with joint deformities, or because of dysesthesias, hyperesthesias or paresthesias.

Typical examples of EM, borrelial lymphocytoma and ACA are illustrated in Fig. 1. More illustrations of these conditions can be found in the previous (1996) publication of European LB case definitions [14].

Nervous system manifestations

Lyme neuroborreliosis (LNB) is mainly an acute disease, which usually develops within a few weeks of infection. In adults, the disease typically presents as painful meningoradiculoneuritis (Garin-Bujadoux-Bannwarth syndrome) and unilateral or bilateral facial palsy. These manifestations may occur separately or in association [10,11]. Radiculitic pain caused by LNB can be severe, but usually decreases rapidly following commencement of appropriate antibiotic

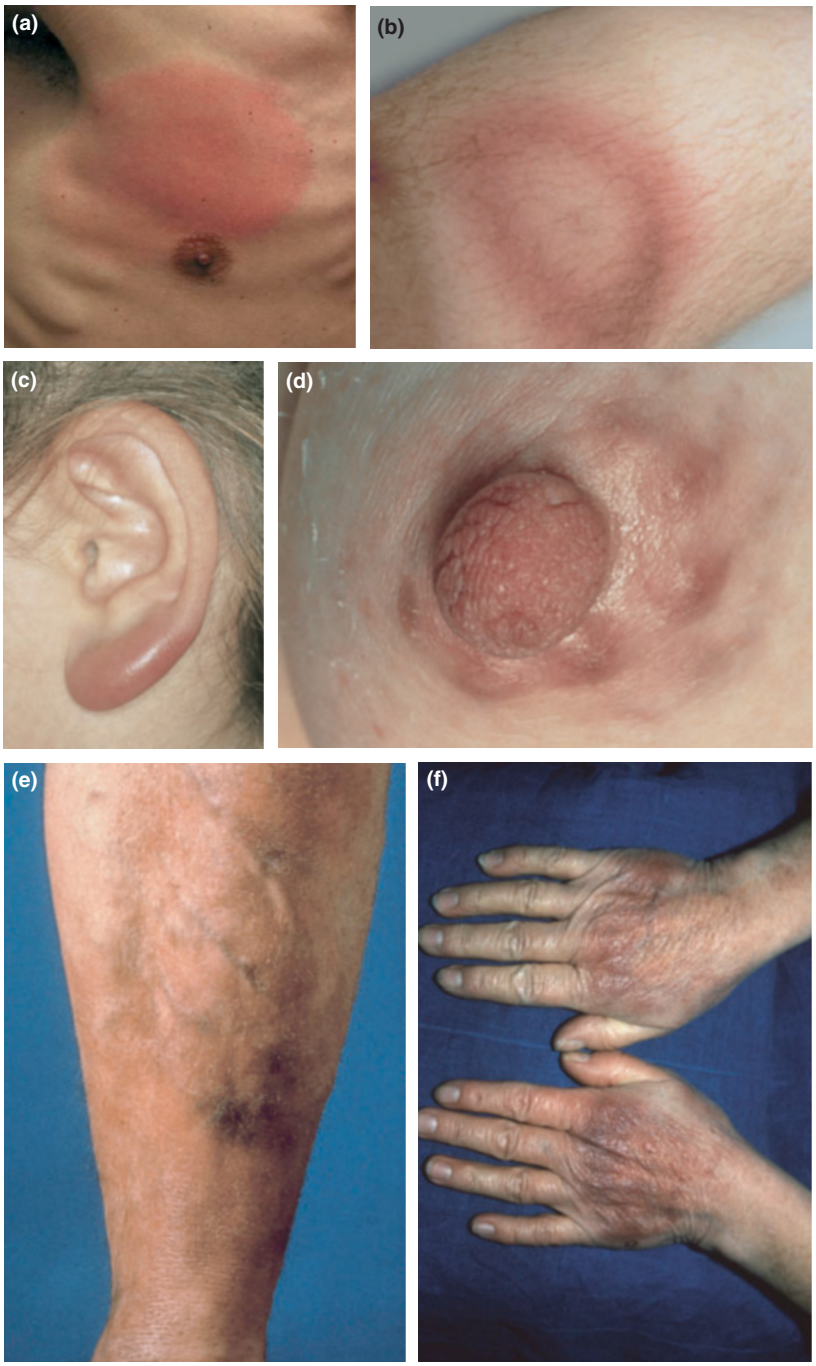

FIG. 1. Skin manifestations of Lyme borreliosis (pictures by *Cortesy of* Hasel Druck und Verlag GmbH, 1090 Vienna, Austria). (a) Erythema migrans on the left breast; about 4 weeks after a tick-bite on this site and 3 weeks after onset of the lesion. (b) Erythema migrans on the lower leg; note central clearing. (c) Borrelial lymphocytoma on the ear lobe. (d) Borrelial lymphocytoma on the nipple. (e) Acrodermatitis chronica atrophicans on the left leg. (f) Acrodermatitis chronica atrophicans on the dorsal side of the hands.

treatment. Less frequent manifestations include other cranial neuropathies involving the VI cranial nerve, less frequently the IV or III and occasionally others. Isolated meningitis in adults, myelitis, encephalitis, cerebral vasculitis presenting as stroke are other rare manifestations. In childhood the most frequent symptoms and signs are headache due to meningitis, and facial palsy [35]. A variety of other neurological manifestations have been described, mostly as single case reports, but very few have been proven to be caused by borreliae. For a reliable diagnosis of LNB, indicative clinical manifestations must be associated with inflammatory cerebrospinal fluid (CSF) parameters, usually including lymphocytic pleocytosis, although pleocytosis in early LNB is occasionally absent. IgM and/or IgG antibodies to *B. burgdorferi* s.l. may be absent in some patients initially, but specific intrathecal IgG production should be detectable in all patients 6–8 weeks

after onset of symptoms [36]. In most cases acute LNB is a self-limiting disease, but some features may persist for months and can result in residual sequelae in a minority of patients even after antibiotic therapy [37]. Peripheral neuropathy as the sole manifestation of LNB is rarely observed in Europe other than in patients with ACA [38].

Long-standing (chronic) borrelial infection of the central nervous system, although very rare, includes long-lasting (at least six months) manifestations such as chronic meningitis, encephalomyelitis, and radiculomyelitis [39,40]. The diagnosis should not be made in the absence of lymphocytic pleocytosis, typically with activated B-lymphocytes and presence of intrathecally synthesized specific IgG antibodies in CSF [14]. Further supporting CSF findings include elevated total protein and oligoclonal bands.

Musculoskeletal manifestations

Lyme arthritis. Manifestations of Lyme arthritis in Europe, as in North America, comprise recurrent attacks or long-lasting objective joint swelling (synovitis), usually in one or a few large joints most commonly the knee [14]. If left untreated the condition may persist for months or even years. Arthralgia, myalgia or fibromyalgia syndromes alone are not criteria for musculoskeletal involvement in LB. Spondyloarthritis, for example sacroiliitis, is not a manifestation of Lyme arthritis. Likewise, polyarthritis of small joints is very atypical for LB, and other differential diagnoses, such as rheumatoid arthritis, must be considered first.

There is no single laboratory marker for the diagnosis of Lyme arthritis. High levels of IgG antibodies to *B. burgdorferi* s.l. are found in serum from patients with Lyme arthritis, but positive serology alone is not sufficient for confirmation, especially in regions where there is high background seroprevalence. In order to substantiate a diagnosis of Lyme arthritis, alternative explanations for the arthritis should be excluded, for example osteoarthritis, trauma, spondyloarthritis, crystal-induced arthritis, and septic arthritis. Synovial fluid analysis is recommended when feasible. Granulocytic inflammation in synovial fluid is a characteristic microscopy finding, and direct detection of *B. burgdorferi* s.l. by culture or PCR in synovial fluid or membrane is highly supportive of a diagnosis. Lyme arthritis is sometimes accompanied by bursitis and/or enthesitis [41], and myositis has also been proven as a rare manifestation of LB [42]. Lyme arthritis is one of the rare inflammatory joint diseases in which routine laboratory parameters of inflammation, such as C-reactive protein levels and erythrocyte sedimentation rate, are often normal. Pronounced elevation of laboratory markers of inflammation in a patient with arthritis argues against Lyme arthritis.

Cardiac manifestations

Cardiac manifestations in LB appear to be rare. Most frequently they can be observed with or shortly after an EM, or in association with neurological symptoms or arthritis. Conduction abnormalities with varying degrees of atrioventricular conduction defects are typical manifestations [10,43]. In particular, Lyme carditis should be suspected in younger individuals showing conduction abnormalities without other apparent risk factors, and who have a history of recent exposure to ticks. Other rhythm disturbances, endomyocarditis and pericarditis have also been reported [44,45]. Alternative explanations for the cardiac condition presented must be excluded. Palpitations, bradycardia, or bundle branch block alone are not sufficient for diagnosis. Antibodies to *B. burgdorferi* s.l. should be evident in serum but positive serology alone is not confirmatory and appropriate clinical signs must also be taken into account.

Chronic cardiac conditions, such as long-lasting dilated cardiomyopathy, have occasionally been associated with borrelial infection but the causal relationship is still unproven despite direct detection of borreliae from endomyocardial biopsies in single cases [46].

Ocular manifestations

The recognition of LB as a potential cause of ocular conditions is important for the implementation of appropriate treatment, although reliable diagnosis is difficult to obtain.

Ophthalmic changes are apparently rare and usually present as conjunctivitis in the course of early manifestations of LB. Uveitis (anterior, intermedia, posterior and panuveitis), papillitis, keratitis, and episcleritis may occur occasionally [47,48].

The differential diagnosis of ocular LB is broad and there is no single laboratory marker for confirmation [49]. Diagnosis requires demonstration of specific serum antibodies and the exclusion of other causes. Evidence for other manifestations of LB in recent medical history and pathological findings in the CSF may further support the diagnosis. If ocular fluid is taken, testing for the presence of borrelial DNA by PCR should be considered.

Objective Long-term Sequelae of LB

Objective long-term sequelae in properly treated patients are uncommon. Some patients, diagnosed in accordance with the case definitions, can take several months to recover fully following appropriate treatment. Similar slow resolution of symptoms and signs can occur in patients with many other systemic infections [50]. Even if some symptoms persist, for

example after Lyme arthritis, additional long-term manifestations such as ACA or late LNB apparently do not develop.

Erythema migrans: There is no evidence for objective long-term sequelae in patients with EM after appropriate treatment [51].

Lyme neuroborreliosis: Recovery from meningoradiculoneuritis and facial palsy may take several weeks to months and will be complete for most patients, except for a small number who may suffer from residual paresthesias or facial paresis. However, in the early weeks of recovery a considerable proportion of patients complain about an inability to work due to neurasthenic symptoms and a reduced tolerance to sustained stress. In rare cases, when the diagnosis of LNB is made late in the course of disease, recovery from severe neurological symptoms may be incomplete (i.e. paresis, hearing deficits, ataxia, incontinence, cognitive impairment) [39,52].

Acrodermatitis chronica atrophicans: atrophic lesions, peripheral neuropathy and joint deformities may remain in patients who sustained severe tissue damage prior to treatment [31].

Lyme arthritis: most patients recover completely but it may take many months. In a small proportion of treated patients (less than 10%) Lyme arthritis takes a more prolonged course, does not respond to further antibiotic treatment and shows no laboratory evidence (culture or PCR) of persistent infection. In these cases arthritis is probably driven by immuno-pathological mechanisms and such patients should be treated with local or systemic anti-inflammatory agents for symptomatic relief and to hasten resolution of the inflammatory response [53].

Subjective Long-term Sequelae of Lyme Borreliosis

Some patients report ongoing, recurrent or persistent symptoms after appropriate treatment of a proven manifestation of LB. This problem, described as post-Lyme syndrome (PLS) [54], is characterized by the persistence of a complex of symptoms for more than 6 months after treatment. The symptoms are nonspecific and include reduced performance, increased fatigue, irritability, emotional lability, and disturbances in sleep, concentration, and memory. Thorough clinical and laboratory assessment of such patients is required to exclude the possibility of treatment failure or the presence of a new condition unrelated to previous LB. Various double-blind, placebo-controlled studies have so far failed to support the idea that persistence of borrelial infection is the cause of such symptoms and have failed to show any sustained benefit from prolonged treatment with antibiotics [55–60]. PLS is sometimes equated with persistent *B. burgdorferi* s.l. infection and referred to as 'chronic' Lyme disease, but this is a misnomer and PLS does not warrant the use of expensive and potentially dangerous antibiotics [61]. For such patients symptomatic treatment is recommended.

Congenital Infection

Despite early suggestions that LB might contribute to unfavourable outcomes in pregnancy [62,63], subsequent studies have not found such an association and good evidence for congenital infection is also lacking [64,65].

Laboratory Diagnosis

LB diagnosis should be based primarily on the clinical presentation and an assessment of tick-exposure risk. In most cases laboratory support is essential because of the nonspecific nature of many clinical manifestations.

Culture of spirochaetes from patient material is still the gold standard for specificity in the laboratory diagnosis of LB [66–71]. However, due to the low numbers of viable spirochaetes usually present in patient biopsies and the fastidious nature of the *B. burgdorferi* s.l. strains the sensitivity of culture is highly variable, ranging from less than 1% in Lyme arthritis to 70% in EM skin lesions [72,73]. Negative results, therefore, do not exclude active infections. For this reason and also because successful culture demands expertise and specialist culture media that is often unavailable in diagnostic laboratories, culture is not used as first line support for clinical diagnosis but may be useful for confirmation and for uncertain cases.

Serology is usually the first and often the only supporting diagnostic measure to be deployed, because it is relatively easy to obtain samples, laboratory testing facilities are widely accessible, and the tests, despite publicised difficulties and controversy, now show acceptable sensitivity and specificity [1,71,74]. However, the limitations of antibody tests must be appreciated. On the one hand the antibody response in early LB may be weak or absent, especially in EM and early LNB [75,76]. Furthermore, seroconversion in such patients may be absent because early antibiotic treatment can ablate antibody production [7,27]. On the other hand, a positive specific antibody response may persist for months or even years after successful treatment of the infection, so follow-up of antibody titres in patients following therapy is not a reliable approach for monitoring success of treatment [77,78].

LB serology in much of Europe follows a two-step approach, involving an initial screening test (usually ELISA), followed by a western blot for reactive and equivocal samples [71,73,79–81]. Recent serological research addresses whether one-step tests, such as ELISAs using the C6 peptide as antigen, are of sufficient sensitivity and specificity to replace the widely used two-step approach. The presence of several pathogenic genospecies in Europe with variability of immunodominant antigens, together with the slightly lower specificity of the single test approach, may limit successful application of such single tests [82,83].

Serology is indicated in all cases of clinically suspected LB except EM, but the less specific the symptoms, the weaker the *a priori* probability of LB, the lower the predictive value of serological methods [71,76,84]. The probability that a patient with a positive serological test actually has LB (positive predictive value) and the probability that a patient with a negative test does not have the disease (negative predictive value) depends on the performance characteristics of a given assay (sensitivity and specificity) and also on the prevalence of the disease in the population [72,85,86]. The pre-test probability of a patient having or not having LB therefore determines the predictive value of the test result. The significance of test results for antibodies to *B. burgdorferi* s.l. must therefore be interpreted with caution, especially outside endemic areas [85].

Technical problems that contribute to false-negative or false positive results include the adoption of inadequate cut-off levels, the presence of cross-reacting antibodies, false positive reactions caused by some autoimmune diseases and inappropriate interpretation criteria for western blots [66]. Several insufficiently evaluated assays for detection of antibodies to *B. burgdorferi* s.l. are currently on the market and unfortunately there is no independent, clinically oriented, pre-market evaluation system for serological assays servicing the EU as a whole [87].

In patients with LNB, CSF examination is important to demonstrate typical, though non-specific, diagnostic clues such as lymphocytic pleocytosis and inflammatory disturbances of the blood-brain barrier [1,66,88]. The analysis of paired serum and CSF samples obtained simultaneously is key to determining the specific CSF/serum antibody index (AI). A positive AI together with typical signs of inflammation in the CSF confirms a clinical diagnosis of LNB [72,88]. Early on in the course of LNB the specific intrathecal antibody response in the CSF may be positive before seroconversion in the peripheral blood (especially in children), but even in seronegative patients with early LNB, signs of inflammation are regularly observed in the CSF [35]. It should be emphasized, however, that a positive specific AI may persist for years after recovery from borrelial CNS infection, whereas other signs of inflammation tend to resolve within a few months (up to 12) [37,71,89].

Almost all immunocompetent patients with late manifestations (arthritis, late LNB, ACA) show a positive IgG antibody response. The diagnosis of so called 'seronegative chronic Lyme disease' in supposed long-standing infections is highly unsatisfactory, requiring further clinical and laboratory investigations [1,70,81]. Seronegative late LB, if it occurs at all, is extremely rare and there have been only two reported cases of apparently seronegative ACA [90] and one of seronegative Lyme arthritis in immunocompetent patients [91]. There are no reliable reports of seronegative late-stage LNB.

Nucleic acid amplification testing using polymerase chain reaction (PCR) technology greatly assists in the detection and identification of a wide range of fastidious pathogens and can detect low copy numbers of *B. burgdorferi* s.l. [69,75,92,93] However, in European LB the spirochaetemia is transient and spirochaetes are relatively difficult to sample from tissues. Furthermore, detection of DNA by conventional PCR cannot unequivocally establish whether infections are active or not. At present, targets, primers and methods are not standardized, so test results obtained by different laboratories may show significant variability. Despite these drawbacks, in the right hands this technique can offer useful diagnostic support in difficult cases. PCR can detect borrelial DNA in over 50% of synovial fluid samples from untreated patients and even higher levels of detection of DNA in synovial membranes can be achieved [94,95] In patients with EM and ACA, borrelial DNA has been detected in 50–70% of skin biopsies though rarely in their serum [72,93], and in acute LNB patients borrelial DNA has been detected in 15–30% of the CSF samples tested [69,89,93,96] The utility of urine-PCR has been investigated by several groups, but results are contradictory and urine PCR is therefore not recommended for routine diagnosis [69,72,97].

Several diagnostic tests, such as the visual contrast sensitivity test, the lymphocyte transformation test, and CD57+/CD3- lymphocyte subpopulation typing, cannot be recommended for diagnosis of LB because they lack specificity [60,71,98].

Concluding Remarks

Clinical case definitions for LB in Europe, together with required supporting laboratory evidence, are summarised in Table 1.

TABLE 1. Summary of clinical case definitions for Lyme borreliosis

Term	Clinical case definition	Laboratory evidence: essential	Laboratory/clinical evidence: supporting
Erythema migrans	Expanding red or bluish-red patch (≥5 cm in diameter)[a], with or without central clearing. Advancing edge typically distinct, often intensely coloured, not markedly elevated.	None	Detection of *Borrelia burgdorferi* s.l. by culture and/or PCR from skin biopsy.
Borrelial lymphocytoma (rare)	Painless bluish-red nodule or plaque, usually on ear lobe, ear helix, nipple or scrotum; more frequent in children (especially on ear) than in adults.	Seroconversion or positive serology[b] Histology in unclear cases	Histology. Detection of *B. burgdorferi* s.l. by culture and/or PCR from skin biopsy. Recent or concomitant EM.
Acrodermatitis chronica atrophicans	Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities. Initial doughy swelling. Lesions eventually become atrophic. Possible skin induration and fibroid nodules over bony prominences.	High level of specific serum IgG antibodies	Histology. Detection of *B. burgdorferi* s.l. by culture and/or PCR from skin biopsy.
Lyme neuroborreliosis	In adults mainly meningo-radiculitis, meningitis; rarely encephalitis, myelitis; very rarely cerebral vasculitis. In children mainly meningitis and facial palsy.	Pleocytosis and demonstration of intrathecal specific antibody synthesis[c]	Detection of *B. burgdorferi* s.l. by culture and/or PCR from CSF. Intrathecal synthesis of total IgM, and/or IgG and/or IgA. Specific serum antibodies. Recent or concomitant EM.
Lyme arthritis	Recurrent attacks or persisting objective joint swelling in one or a few large joints. Alternative explanations must be excluded.	Specific serum IgG antibodies, usually in high concentrations	Synovial fluid analysis. Detection of *B. burgdorferi* s.l. by PCR and/or culture from synovial fluid and/or tissue.
Lyme carditis (rare)	Acute onset of atrio-ventricular (I–III) conduction disturbances, rhythm disturbances, sometimes myocarditis or pancarditis. Alternative explanations must be excluded	Specific serum antibodies	Detection of *B. burgdorferi* s.l. by culture and/or PCR from endomyocardial biopsy. Recent or concomitant erythema migrans and/or neurologic disorders.
Ocular manifestations (rare)	Conjunctivitis, uveitis, papillitis, episcleritis, keratitis.	Specific serum antibodies	Recent or concomitant Lyme borreliosis manifestations. Detection of *B. burgdorferi* s.l. by culture and/or PCR from ocular fluid.

[a]If <5 cm in diameter a history of tick-bite, a delay in appearance (after the tick bite) of at least 2 days and an expanding rash at the site of the tick-bite is required.
[b]as a rule, initial and follow up samples have to be tested in parallel in order to avoid changes by inter-assay variation.
[c]In early cases intrathecally produced specific antibodies may still be absent.

Case definitions are essential for reliable epidemiological studies and are of great value in clinical management. In clinical studies they can assist in collection and analysis of appropriate clinical and laboratory data and facilitate comparison of findings from different studies. The case definitions described here include basic clinical features and the use of laboratory data, either as supporting or confirmatory evidence, using methods that are well-characterized in the routine diagnosis of LB. Serology and culture remain the cornerstones of laboratory methods for diagnosis of LB. Although detection of *B. burgdorferi* s.l. DNA by PCR is increasingly used in laboratory diagnosis, this method has significant limitations and there is no general agreement on the most appropriate genomic targets for amplification and whether or not positive results are clinically significant in some manifestations of the disease. Other laboratory methods, reported to be potentially helpful, have not been included here since protocols remain essentially non-standardized or their use in a clinical context is not fully agreed.

These updated case definitions are designed to assist clinicians in the accurate diagnosis of LB in Europe, through description of the full clinical spectrum of the disease and recommendations for the use of laboratory support. They may also be used for epidemiological purposes.

Transparency Declaration

No conflict of interest.

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