Chronic Lyme Disease: A Controversy of Definitions and Diagnostics

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Abstract

The question of whether Lyme disease can become a persistent infection in the form of "chronic Lyme disease" has caused heated debates, public protests, litigation, harassment and even death threats over the last decades. Against the established view of Lyme disease as an acute infection that is easily treated with few complications once recognized, a vocal dissenting minority of scientists maintains the existence of an unrecognized epidemic of chronic bacterial infestation caused by the tick-borne Borrelia spirochetes.

We aim to review interpretations of continued reporting of symptoms despite antibiotic treatment as per standard of care; while the term "chronic Lyme disease" implies an unsuccessfully eradicated infection, Post-Lyme disease syndrome accounts for this as post-infectious sequelae. In addition, we also examine diagnostic strategies, summarize the contenting recommendations given by the two sides, and briefly asses some of the more controversial laboratory tests being used for chronic Lyme Disease in the private market.

We find that "chronic Lyme disease" is defined as a very vague diagnosis based on symptoms and clinical findings, of which several are present in more than 10% of the general population, regardless of exposure indicators such as a recognized tick-bite or Borrelia being endemic in the area or not, and supported by a myriad of unvalidated diagnostic tests. However, we also find that existing test strategies suffer from potential selection bias and high variability, and conclude further research towards better discriminatory tests is warranted.

Chronic Lyme Disease: A Controversy of Definitions and Diagnostics

1. The Lyme disease controversy

The controversy surrounding Lyme borreliosis has flared over the last decade, culminating in an embittered debate complete with public protests, legislative actions, a flurry of publications denying and renouncing the opposing side and even harassment and death threats (1). In the U.S. state of Connecticut, where Lyme was first recognized as a tick-borne disease, the Attorney General Richard Blumenthal instigated an anti-trust investigation against the Infectious Disease Society of America (IDSA) for its alleged monopoly over clinical treatment guidelines (2). A 2008 settlement required the IDSA to reassess its guidelines with a new panel vetted by an independent ombudsperson; however, this panel concluded two years later that existing guidelines were sound and that no changes were necessary (3). Locally, public perceptions differ sharply from the view of the established medical community.

A recent article investigating the beliefs of 421 inhabitants of two communities in Connecticut and Rhode Island where Lyme disease is endemic reported more than three-quarters of respondents agreeing with the statement that Lyme spirochetes could persist in the body despite short-course antibiotic treatment, and nearly one-third believing that antibiotics could only palliate symptoms of Lyme disease, not provide a definitive cure (4). In contrast, in a recent survey of primary care physicians in Connecticut, only 2.1% of respondents reported they habitually diagnosed and treated chronic Lyme, whereas 49.8% reported they did not believe in the existence of "chronic" Lyme disease (5). Views of the incidence of Lyme disease have evolved as well. Preliminary estimates released by the Centers for Disease Control and Prevention earlier this year indicate that the number of Americans diagnosed with Lyme disease each year – some 300,000 – may be ten times the number of physician-reported cases, previously considered the best prevalence estimate (6,7). Consequently, it is hardly audacious to assume the growing discussion on the diagnosis and treatment of chronic Lyme infection, whether evidence-supported or not, is unlikely to quiet down, but will remain an important subject for clinicians to follow if they operate in a setting where Lyme disease is a possibility.

In the present article, we aim to introduce the reader to the contrasting views on Lyme disease, briefly summarize the disagreements in diagnostic and therapeutic recommendations, and scrutinize the evidence underlying the most common diagnostic tests.

2. Post-disease sequelae or active infectious process?

Lyme disease is named for the town of Lyme, Connecticut, where a young Yale rheumatologist, Allan C. Steere, investigated a cluster of juvenile rheumatoid arthritis cases in 1975, and subsequently published evidence of the first acknowledged outbreak of a new disease carried by the *Ixodes* tick vector (8,9). Its linkage to the characteristic rash, *erythema chronicum migrans,* was reproted by Arvid Afzelius in Sweden as early as 1909, and identified in a case report in the United States in 1970 where the author, Rudolph Scrimenti, suggested a spirochete or rickettsial microbe transmitted via ticks as vectors (10,11). The causative agent of Lyme disease, a spirochete bacteria, was identified by Willy Burgdorfer at the National Institutes of Health's Rocky Mountain Laboratories and later named for him as *Borrelia burgdorferi* (abbr. *Bb.*) (12). These historical facts may seem to be the only thing about the disease that is not bitterly contested in what has been dubbed as "the Lyme Wars"; symptoms, diagnosis, definitions, prevalence, treatment and the costs to society are all subject to acrimonious dispute in the public realm.

According to the academic medical canon, Lyme disease is caused by transmission of one of the Borrelia spirochetes (typically *B. garinii* and *B. afezelii* in Europe and *Bb. sensu stricto* in the United States) via a bite from *Ixodes scapularis* (Northeast and Midwest United States), *I. pacificus* (Western United States) or *I. ricinus* (Europe). Thus, the disease is geographically restricted according to tick habitats (13-16). Any tick that is infected with one of several different strains of *Bb.* carries the potential to transmit the spirochete, although the risk of transmission is reduced if the tick was attached for <36 hours (13). Estimates vary, but somewhere between 60-80% of patients seem to develop a clinically distinguishable "bull's-eye" skin rash, *erythema migrans* (14,15). Left untreated, the bacteria can disseminate and cause disseminated or late disease with cardiac (atrioventricular block or myopericarditis), neurological (lymphocytic meningitis, facial nerve palsy, and/or encephalitis) or arthritic (polyarthritis migrans) manifestations (16). At least 19 independent medical professional societies representing the United States and most European countries recommend a short course of oral antibiotic; if initiated and followed appropriately, failure of this treatment to eradicate the infection is considered rare, and recurrence of the infection in the absence of an additional suspicious tick bite is considered non-existent (17,18). Any remaining symptoms are ascribed to post-infectious sequelae resulting from tissue damage (19).

However, according to the dissenting view, *Bb.* is a bacteria than can survive as an ongoing infection even after acknowledged treatment and become the source of chronic illness (20). The International Lyme and Associated Diseases Society (ILADS) was formed in 1999 for the purpose of promulgating this dissenting conceptualization of Lyme disease. ILADS argues that Lyme disease "knows no borders" and is widespread around the world; that symptoms are broader than the case definition; that those often go unrecognized by clinicians; that testing is inaccurate; and, perhaps most controversially, that short-course antibiotic treatment can and does fail in large number of patients that continue to suffer persistent symptoms unless treated with prolonged antibiotic therapy aimed at eradicating the presumed surviving spirochetes (21). Criticism leveled by ILADS at the mainstream medical establishment and its recommendations for Lyme disease has been passionate and at times vitriolic. For example, Raphael Stricker, physician-scientist and past president of ILADS, has challenged the conclusions of the review panel convened as per the 2008 settlement on grounds of bias; although vetted by an ethicist (paid by the IDSA) for conflicts of interests, Stricker claims the panel failed to be independent due to failure to consider the organizational membership of its members (7 of 8 were members of the IDSA) and the selection of a IDSA past president as its chair (22). Temperatures in the discourse between "Lyme activists" and proponents of the established medical perspective have been heated for years, with accusations of pseudoscience, flawed science, scientific dishonesty, corruption and conspiracy being exchanged repeatedly (1,23,24).

Post-Lyme disease syndrome

Reports of symptoms persisting for years after completion of antibiotic therapy have persisted since the early 1990s (25). A 2005 meta-analysis consisting of five studies with a total of 504 patients and 530 controls with a mean or median time since onset of 3-6 years found significantly higher prevalence of fatigue, musculoskeletal symptoms (joint or muscle pain, aches, and swollen joints) and neurocognitive difficulties (including memory problems, poor concentration, and expressive aphasias) in patients with a history of treatment for Lyme disease versus healthy controls (26). Two post-treatment syndromes have been distinguished; the rarer one is characterized of persistent large-joint synovial inflammation, and is proposed to be caused by molecular mimicry between a spirochete outer surface protein (OspA) and an adhesive molecule expressed on T-cell surfaces (LFA-1) resulting in cross-reactivity and subsequent persistent autoimmune arthritis (27). However, far more commonly residual symptoms are thought of as due to late onset of antibiotic treatment and slower therapy response, causing irreversible tissue damage (28). Absent alternative explanations, such as comorbidities that can explain persistent

manifestations (e.g. chronic fatigue syndrome, fibromyalgia, multiple sclerosis, or neuropathies), the latter syndrome can be classified as "Post-Lyme disease syndrome" (17). Studies have shown that patients who presented with disseminated disease and severe illness, experienced delayed antibiotic treatment or were of adult age are more likely to suffer from Post-Lyme disease syndrome, while the duration of the antibiotic treatment was not associated with added risk (29). As the syndrome is attributed to irreversible damage and not an active infection process, laboratory tests such as culture growth, serologic testing, and PCR assays, are not expected to be helpful unless it is to rule out other causes.

Not even the concept of symptoms persisting beyond treatment is uncontroversial; Shapiro et al. claim the only meta-analysis on post-Lyme disease syndrome is "uninformative and misleading" (30). However, some of the arguments the authors put forth in support of their conclusions are irrelevant (untimely or inadequate antibiotic therapy in early studies), others can hardly be said to rule out the possibility persistent symptoms (study designs that were retrospective, patients with extra-cutaneous manifestations were overrepresented), while some requires serious consideration (testing procedures and seropositivity in disease). Also, a prospective population-based study 114 serology-confirmed cases of neuroborreliosis diagnosed according to the CDC surveillance criteria suggested high rates of residual symptoms even after antibiotic treatment at the 5 year follow-up, with 25% still suffering from symptoms that had occurred when they were first diagnosed with the disease (31).

Chronic Lyme syndrome

Beyond the common belief among proponents of chronic Lyme disease in a persistent infectious process where the spirochete escapes detection by the immune system, there is no precise, consistent case definition that is shared among the advocates of this disease entity. Reported symptoms have included manifestations in almost any part and organ system of the body, with a preponderance of symptoms involving the central nervous system, (brain, spinal cord, nerve roots and peripheral nerves), joints, skin, heart, and eyes (32). According to ILADS, patients can get infected with *Bb.* and remain seronegative by conventional laboratory tests (i.e. remain below the IgM and IgG ELISA and WB band cut-offs), but still progress to illness (33). Global prevalence of Lyme disease is contended to be significantly higher than that reported to the CDC, claiming that the CDC-recommended testing strategy leaves >90% of patients undetected and that the prevalence of Lyme disease is far less geographically restricted than traditionally proposed (33).A comprehensive observational assessment published by Ljøstad in 2012 examined 29 patients with "chronic Lyme disease", included on the basis of their doctor suspecting an

ongoing *Bb.* infection despite treatment and recruited through various channels, including public media, found the most predominant symptom in their patient group to be fatigue, and then in falling order pain, walking impairment, memory/concentration problems, headache, itching and dizziness (34). No evidence of ongoing *Bb.* was found in any patient even after a wide array both normal clinical bloodwork and diagnostic tests for Lyme disease, sometimes in parallel using kits from multiple manufacturers. Eight (28%) were found to have other well-defined illnesses, while fourteen (48%) had presence of *anti-Bb.* IgG antibodies. Twenty-one (72%) had symptoms of unknown cause, of those six met the suggested criteria for post-Lyme disease syndrome. These six patients had more fatigue and poorer health-related quality of life as compared to controls, but were not more depressed, anxious, or hypochondriac in general. Their beliefs about the illness were characterized by negative expectations.

Molecular diagnosis of Lyme disease

The most widely acknowledged diagnostic strategy for Lyme disease is a two-tier testing process; on initial suspicion, a sonicated IgM and IgG enzyme immunoassay (ELISA) is conducted which - if positive - is followed by a confirmatory Western immunoblot (WB) (35). If present by WB, an ELISA test is considered to have a sensitivity of 95-100% and a specificity of 95% (ref). In 2008, Steere et al. conducted the first prospective study of these serologic tests; they examined 76 cases of culture-confirmed erythema migrans (40 with disseminated disease, as determined by the presence of more than one skin lesion) and 44 IgM/IgG seropositive cases of extracutaneous manifestations against 136 healthy controls indicate suggested low sensitivity of testing in the acute-phase of erythema migrans (17% with solitary lesion, 43% with disseminated (multiple lesions) disease), substantially higher in the convalescence 3-4 weeks later (43% with solitary lesion, 75% with disseminated disease) and a 100% sensitivity for neurological, heart and joint symptoms consistent with late borreliosis (36). Specificity of the two-tier testing protocol was found to be 99% at all levels.

More recent studies show that the *Bb*. bacteria in vivo express certain antigens such as the C6-peptide, which is a part of the lipoprotein VlsE in the outer membrane of the spirochete (37–40). Steere also demonstrated that by adding ELISA-testing for IgG VlsE C6 peptide ELISA (overall sensitivity 29% for the acute-phase, 56% for convalescence and 100% for extracutaneous manifestations; specificity of 96% for all stages) sensitivity could be improved in the early phase of disease (36). It is important to note that the high sensitivity for late manifestations must be considered artificially elevated; there was no "gold standard" for definitive confirmation of disease, and a positive IgM or IgG antibody response served as inclusion criteria for all patients without an erythema migrans. The authors acknowledge the problem of

a assessing sensitivity in a reference population where having a previous positive test is a requirement for being included as a case; however, they justify this by arguing that all existing studies support the assumption that all patients with objective neurologic, cardiac, or joint abnormalities associated with Lyme disease will also have seropositivity on serologic tests.

Based on these data, the CDC recommends the two-tier protocol of ELISA followed by a confirmatory WB. For patients who present without erythema migrans, serologic testing should be considered insensitive; thus, the means of diagnosis is based on inspection of the skin lesion and treatment based on clinical judgement (41). As for patients who presents symptoms from a later stage of the disease, such as signs of neuroborreliosis (Bannwarth's syndrome/meningopolyradiculitis; radiculopathy; Bell's paresis, facial palsy and other cranial neuropathies; lymnphocytic meningitis or encephalomyelitis), cardiac manifestations (intermittent atrioventricular block, myopericarditis), and newly onset mono- or polyarthritis the recommendation is that they should carefully be examined for skin lesions and have two-step testing (42–44). When patients have neurological manifestations, IDSA guidelines recommend they should also be tested for CSF antibodies to *Bb.* (45).

However, using antibodies as the mainstay of the diagnostic strategy does have its problems. Specific antibodies found in a sample can originate from activation of the immune system years before the sample was taken and have no relation to the symptoms the patient may have, acting more as a 'serological scar'. In southern Norway, around 18% of blood donors has been shown have IgG antibodies against Borrelia (46). Many patients in the initial stadium of the disease do not produce a detectable antibody response; only 43% of those with a culture-confirmed solitary erythema migrans lesion will create a measurable IgM or IgG response 3-4 weeks after the onset of symptoms (37,40,47).

The issue of diagnostic sensitivity and the need serological proof of infection has become a heated subject of controversy. While ELISA sensitivity is often reported to be high in the literature, the IDSA guidelines only cite a limited set of studies as evidence, either directly or indirectly (48). A study by Aguero-Rosenfeld in 1993 prospectively assessing 100 erythema migrans found 35% and 44% to be seropositive by ELISA and IB at the time of presentation; seroconversion one month later was seen in 28 of 38 (75%) and 21 of 33 (64%) by ELISA and IB, respectively (49). Multiple studies in the 1990s estimated sensitivity to be anywhere from 70 to 95% for later stages of Lyme disease, such as Lyme arthritis and acrodermatitis chronica atrophicans (50). A 1996 study with of 111 patients with case definitions based on clinical criteria reported 100% sensitivity in later Lyme stages; however, the authors admit that physicians recruiting to the study where practiced in Lyme-endemic areas, and referring

physicians would have had access to the result of other serologic tests performed as part of clinical practice before submitting patients for inclusion, allowing for selection bias in favor of seropositive study participants (51). A recent study also demonstrated considerable discordance between eight available ELISAs and five immunoblots used commercially, resulting in widely divergent sensitivity and specificity when analyzing different ELISA-immunoblot combinations using the two-tier algorithm (52). Examining 89 patients and controls, only 18% of samples were positive in all ELISAs and 43% were positive in anywhere from one to seven kits from various manufacturers.

Diagnosis of chronic Lyme disease

Citing problems with the conventional diagnostic strategies for Lyme disease, ILADS has released its own and markedly different set of guidelines for diagnosis and treatment of Lyme disease. ILADS purports that chronic Lyme disease should be diagnosed on the basis of persistent symptomatology that includes subjective findings of fatigue, headaches, and sleep disturbance, as well as objective neurologic and neuropsychiatric features (e.g. cognitive dysfunction, demyelinating disease, peripheral neuropathy, motor neuron disease), cardiac presentations (e.g. electrical conduction delays and dilated cardiomyopathy), and musculoskeletal problems. These symptoms despite internationally recommended treatment, with potential relapses in the absence of another tick-bite or erythema migrans rash (recurrent Lyme disease), or be poorly responsive to antibiotic treatment (refractory Lyme disease) (33). The diagnosis is presented as primarily clinical, with laboratory tests only supporting infection and never excluding it.

According to the newest (2004) ILADS guidelines, Lyme disease should be suspected in patients with one or more of newly acquired or chronic (terms are both undefined) fatigue; fevers, 'hot flashes' or chills; night sweats; sore throat; swollen glands; stiff neck; migrating arthralgias; stiffness and, less commonly, frank arthritis; myalgia; chest pain and palpitations; abdominal pain; nausea; diarrhea; sleep disturbance; poor concentration and memory loss; irritability and mood swings; depression; back pain; blurred vision and eye pain; jaw pain; testicular/pelvic pain; tinnitus; vertigo; cranial nerve disturbance (facial numbness, pain, tingling, palsy or optic neuritis); headaches; 'lightheadedness' or dizziness. As the guidelines advocate the use of a more than thirty symptoms commonly expressed in the general population, it should be no surprise that studies demonstrate that a considerable subset of those with long-standing complaints ascribed to Lyme had an alternate medical diagnoses (20-39%) or post-Lyme disease syndrome (13-20%) (34,53–55). Although there are some reports suggesting that Chronic

Fatigue Syndrome and fibromyalgia may be triggered by Lyme borreliosis, this is also true for in the aftermath of other infections (56).

Prophylaxis and treatment recommendations

A 2010 meta-analysis of four randomized clinical trials found antibiotic prophylaxis after tick-bites to be effective, with the largest of them (506 patients) demonstrating a significant treatment effect using a single-dose regimen of 200 mg doxycycline (13,57). Amoxicillin can be substituted for doxycycline if the patient is pregnant or <8 years old, but prophylaxis is only recommended in areas where the b. burgdorferi prevalence among ticks is >20 % (48). Internationally, doxycycline (100 mg twice per day), amoxicillin (500 mg 3 times per day), or cefuroxime (500 mg twice per day) for 10-21 days is recommended as treatment for adult patients with localized disease associated in the form of erythema migrans or generalized Lyme arthritis; the Scandinavian countries, another region where borreliosis is endemic, favor the use of penicillin V (1-1,3 g 3 times per day) as a first choice for erythema migrans alone (18,48,58). For neuroborreliosis, the most common choice has traditionally been ceftriaxone (2 g daily for 14-28 days), although more recently prepared guidelines have started recommending doxycycline for neuroborreliosis without encephalitic or myelitic features, following publication in 2008 of a Norwegian double-blind randomized controlled trial which showed non-inferiority of oral doxycycline 200mg daily versus intravenous ceftriaxone 2g daily for 14 days (59). Based on four randomized controlled trials, the standard of general medical practice also states that long-term intravenous antibiotics have no role in the treatment of post-disease sequelae (60–62).

The ILADS guidelines recommend that antibiotic treatment should depend on severity and be individualized (33). Oral amoxicillin, azithromycin, cefuroxime , clarithromycin, doxycycline and tetracycline are all recommended as more or less clinically equivalent first-line agents at the same dosages as IDSA guidelines for treatment of early Lyme borreliosis. However, for chronic Lyme disease the ILADS advocate sequential treatment (intravenous therapy e.g. with ceftriaxone and then convert to oral treatment for 'persistent or recurrent Lyme disease'), they suggest vastly higher doses of oral agents (e.g. 3000-6000 mg amoxicillin, 300-400 mg doxycycline and 500-600 mg of azithromycin). Because of the perceived failure of shorter-term courses of antibiotics "the practice of stopping antibiotics to allow for a delayed recovery is no longer recommended for patients with persistent, recurrent and refractory Lyme disease". Instead, the antibiotic regiment should be individualized, any relapse should be met with prompt and aggressive treatment, and the decision to stop antibiotics should be based on multiple factors, among them the probability of refractory Lyme disease in the case

at hand and the importance of discontinuing antibiotics. Thus, therapy for years with antibiotics would be within the ILADS guidelines if the person was considered to have refractory Lyme.

3. Other proposed tests for Lyme borreliosis

A case example of a chronic Lyme laboratory

As a case example of we examined the range of services offered by Borreliose Centrum Augsburg (BCA-Clinic), a large private 'Lyme specialty clinic' in Northern Europe which advertises itself as a center for tick-borne diseases and also runs Infectolab, a reference laboratory for other 'Lyme specialty clinics' in large part of Northern Europe headed by Armin Schwarzbach, who is also the chairman of the Laboratory and International Committee of ILADS. According to its brochure, BCA-Clinic performs

1. Borrelia IgM/IgG-EIA and –immunoblot (incl. VIsE) tests;
2. Direct detection by PCR;
3. Lymphocyte transformation tests (LTT);
4. CD3-/CD57+ expression tests;
5. Differential diagnostic tests in the form of ordinary biochemical organ profiles, markers for autoimmunity, serology for alternate infectious causes (e.g. herpes simplex, Epstein-Barr virus and toxoplasmosis), serology/LTTs for 'tick-borne coinfections' (Ehrlichia/Anaplasma, Babesiosism, Rickettsia, Bartonella, chlamydia and mycoplasma) and differential procedures considered beyond the scope of this test.

Direct detection by PCR

When PCR assays were first introduced, hopes were that they would prove an improvement of direct culture of Bb given that the number of spirochetes in infected tissues or body fluids of patients often remains low (63). Although direct cultivation in a Barbour-Stoenner-Kelly medium is possible, its sensitivity is largely undefined and the method can require up to 12 weeks of incubation before being considered negative. Unfortunately, PCRs has proven less sensitive than anticipated; a review found a median sensitivity of 14% for blood, plasma and serum (6 studies) and 38% for cerebrospinal fluid (16 studies) (63). Median sensitivities were somewhat better when PCR was used on skin biopsies from erythema migrans (69%; 16 studies) or acrodermatitis chronica atrophicans (76%; 8 studies) and synovial fluid from arthritic joints (78%; 8 studies) (63). A recent publication also reported a PCR assay aimed at overcoming the low number of spirochetes in whole blood which seemed to have higher sensitivity (62%) – which rose to 90% when combined with two-tier testing - for early Lyme in 21 patients (64).

Lymphocyte transformation tests

Lymphocyte transformation tests (LTTs) are built on the principle that T-cells who recognize Borrelia-specific antigens will transform to an active state with subsequent proliferation and cytokine production. Using radioactive isotopes, increased DNA synthesis is used as a measure of activation as done in the LTT MELISA. The idea was first published by Stejskal in 1994 as a test of hypersensitivity towards various metals, but has later been criticized (including by a co-author on the original article) for low specificity (65–67). Valentine-Thon studied 244 using an LTT protocol which found 86,2% of LTT-MELISA positive patients to be seropositive, claiming most patients (90.7%) showed negative or markedly reduced lymphocyte reactivity after treatment (68). Most guidelines have pointed out that these results had not been independently validated; however, another group recently reported similar results in a study of 302 patients of known serological status with LTT sensitivity of 89,4% before antibiotic treatment and a specificity of 98,7%, again followed by subsequent return to negative tests following antibiotic treatment (69). A related LTT ELISpot (enzyme-linked immunospot) test using nitrocellulose membranes and epitope-specific monoclonal antibodies has been adapted for Lyme, but the studies make clinical sensitivity hard to assess and have yet to be validated by other groups (70–72).

CD3-/CD57+ T-lymphocytes

Stricker et al. has reported results obtained by flow cytometry using antibodies against specific T-cell markers that show reduced subsets of CD3-/CD57+ Natural Killer (NK) cells in 73 'LTT-verified' chronic Lyme cases compared to 10 acute Lyme and 22 AIDS patients (73). Shortly after, the same authors published more studies; one study followed one patient with purported chronic Lyme over a decade and one focused on musical hallucinations in two more patients, all showing attenuated populations of CD3-/CD57 NK-cells (74,75). The authors claim the test can both support a diagnosis of chronic Lyme and be used to measure treatment effect as patients undergo courses of antibiotics as per ILADS recommendation. However, in 2009 Marques performed immunophenotyping in 9 individuals with post-Lyme disease syndrome, 12 who recovered from Lyme disease, and 9 healthy volunteers; no statistical difference was found between the groups (76). In addition to not being independently reproduced, the Office of Research Integrity at the National Institutes of Health examined Stricker in 1993 for scientific misconduct, and concluded that he had selectively suppressed data, which led to retraction of an HIV-related article in the New England Journal of Medicine (1).

4. Conclusions

Is there merit to the notion of chronic Lyme as a clinical manifestation of a persistent infection, or does any remaining symptoms after treatment consist of sequelae due to tissue damage from an earlier infection? From the turn of the millennium, studies investigating diagnostic sensitivity started incorporating seropositivity to various extents as part of their case definition; patients are enrolled in studies only if they have already tested positive on another serologic test, which creates a selection bias skewing the population towards a case subset with more reactive sera and produce falsely high sensitivities (36,77,78). As previously discussed, both interlaboratory and test kit variability have been notoriously variable since it was first measured, and as recently as 2011, a study comparing eight commercial ELISAs together with five immunoblots found positive results to range anywhere between 34 to 59% among 89 patients, and some immounoblots gave positive results in samples tested negative by all eight ELISAs (52,79,80). Obviously, they cannot all be correct at the same time; with such discordance between tests the statement that the purported sensitivity with the two-tiered protocol is high must be viewed as misleading.

We know little of the true sensitivity of modern ELISA and IB assays, because seropositivity at the time of study enrollment has more or less become an informal requirement in all studies over the last decade, despite the fact a share of patients failed to seroconvert in early prospective studies of erythema migrans patients without a definite reason being established for this (49,81). In practice, seroconversion by ELISA and IB are used as the gold standard when selecting true positives, creating a built-in bias to select the patient subset with more reactive sera. To obtain a better estimate of how modern ELISA and IB assays perform in clinical practice, both extensive standardization work and a return to a diagnostic case definition that does not simultaneously use the characteristic tested for as its own gold standard.

Two decades after the idea of Lyme disease becoming a persistent infection was floated, definite molecular evidence conclusively demonstrating this has yet to be published. However, even if the *Bb.* spirochete is indeed a master of stealth, capable of playing hide-and-seek with the immune system using cystic forms and various other molecular dazzles, it seems an unlikely scientific explanation for most people claiming to suffer from the illness. The criteria put forth by the ILADS are of exceedingly vague and non-specific, several of them present at more than 10% of the general population regardless of whether Lyme disease is endemic in the area or not (82). Combining a list of 33 symptoms, many highly expressed at baseline in the general population, with a multitude of diagnostic tests will introduce a

multiple comparisons problem in itself; when several of the more controversial diagnostic tests offered on the private market rest on what cannot be called other than a marginal evidence base showing limited sensitivity and specificity, statistical reasoning suggest their positive predictive value will be low. This applies even more so if such tests were used to screen a population selected not on the basis of clinical probability of Lyme, but as part of a more unspecific hunt for causes behind common symptoms such as fatigue, abdominal pain, headache and sleep disturbance.

While diagnostic tests may perform poorer due to selection bias than generally perceived in the medical literature, available evidence from prospective studies done before seropositivity became a requisite for inclusion does not suggest vast numbers of cases of Lyme borreliosis going undetected; more likely, extensive use of diagnostic tests for common complaints will lower the positive predictive power and result in a larger share of false positives. Considering that several of the more controversial tests used by 'Lyme specialty clinics' rest on sparse evidence and has not been independently reproduced, scientific jurisprudence suggest such tests should not be employed in routine diagnostics until more research support their use.

The two-tiered testing protocol in patients with symptoms suggestive of Lyme disease may be sensible strategy to limit the proportion of misdiagnosed patients, but has multiple limitations. Its sensitivity is low in early Lyme disease and may be artificially high in later stages due to the use of the same methodology as a gold standard. In endemic areas seroprevalence may be as high as 15-20% and a substantial subset of patients presenting with long-standing complaints ascribed to Lyme borreliosis (29-48%) also have antibodies against Bb, but the current test regime has no reliable way of discerning acute borreliosis from an successful treated case of Lyme disease, an unsuccessful treatment attempt or a potential re-infection (34,53). As the controversy surrounding Lyme disease display no hint of tapering down, continued research into new and more precise diagnostic tools decidedly seems warranted.

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