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REVIEW

Chronic Lyme disease: misconceptions and challenges for patient management

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Abstract: Lyme disease, infection with the tick-borne spirochete *Borrelia burgdorferi*, causes both specific and nonspecific symptoms. In untreated chronic infection, specific manifestations such as a relapsing large-joint oligoarthritis can persist for years, yet subside with appropriate antimicrobial therapy. Nervous system involvement occurs in 10%–15% of untreated patients and typically involves lymphocytic meningitis, cranial neuritis, and/or mononeuritis multiplex; in some rare cases, patients have parenchymal inflammation in the brain or spinal cord. Nervous system infection is similarly highly responsive to antimicrobial therapy, including oral doxycycline. Nonspecific symptoms such as fatigue, perceived cognitive slowing, headache, and others occur in patients with Lyme disease and are indistinguishable from comparable symptoms occurring in innumerable other inflammatory states. There is no evidence that these nonspecific symptoms reflect nervous system infection or damage, or that they are in any way specific to or diagnostic of this or other tick-borne infections. When these symptoms occur in patients with Lyme disease, they typically also subside after antimicrobial treatment, although this may take time. Chronic fatigue states have been reported to occur following any number of infections, including Lyme disease. The mechanism underlying this association is unclear, although there is no evidence in any of these infections that these chronic posttreatment symptoms are attributable to ongoing infection with *B. burgdorferi* or any other identified organism. Available appropriately controlled studies indicate that additional or prolonged courses of antimicrobial therapy do not benefit patients with a chronic fatigue-like state after appropriately treated Lyme disease.

Keywords: Lyme disease, *Borrelia burgdorferi*, chronic, diagnosis, treatment, chronic fatigue, neuroborreliosis

Background

The debate about "chronic Lyme disease" provides a remarkable example of how heated a conversation can become when people use words differently. Contested issues largely stem from very different understandings of what terms mean. The broad medical and scientific communities use the term "Lyme disease" to refer specifically to infections with *Borrelia burgdorferi* and closely related European *Borrelia* spp. Proponents of the concept of chronic Lyme disease, who typically refer to themselves as "Lyme literate", use the term to refer to a constellation of disabling symptoms that may or may not be related to this or other infections they believe to be tick transmitted. This clinical syndrome largely overlaps with the disorder commonly known as "myalgic encephalomyelitis/chronic fatigue syndrome", or as an Institute of Medicine committee recently recommended, "systemic exertion intolerance disease" or SEID[1] – a disorder

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that is real, disabling, and may appear to develop following an infectious illness.

The medical/scientific community uses the term "nervous system Lyme disease" to refer to disorders in which there is objective evidence that this organism has physically invaded the nervous system and the infection, or the host response to it, is having a specific impact on neurologic function. The "Lyme literate" use the term to include a broad array of neurobehavioral phenomena, with no requirement of objective evidence of actual nervous system infection. The medical/scientific community uses the term "chronic Lyme disease" to describe individuals with objective evidence of longstanding ongoing infection, while the "Lyme literate" use this term to describe individuals with chronic, life-altering symptomatology without necessarily having biologic evidence of persisting infection. This might be considered little more than a semantic debate (as Humpty Dumpty famously said,[2] "A word shall mean exactly what I choose it to mean, neither more nor less, it's merely a question of who's to be the master"). However, since the "Lyme literate" construct is used to justify prolonged courses of antimicrobial therapy with significant potential for complications, impact on community antimicrobial resistance, and consumption of health care resources, it is essential that the terms be defined with clarity.

Introduction – can Lyme disease cause chronic infection?

In 1977, Steere et al[3] described a syndrome including tick bites, a rash termed at that time erythema chronicum migrans (now erythema migrans, EM), nonspecific symptoms including headache, malaise, fatigue, myalgias, and fever, and recurrent episodes of frank arthritis, with disease duration of up to 22 weeks. In these authors' subsequent description of effective treatment of Lyme meningitis,[4] meningeal symptoms developed on an average of 5 weeks (but up to 12 weeks) after initial evidence of the infection; patients were initially evaluated by the authors at a mean of 6 weeks (up to 12) after initial neurological abnormalities, and then were treated successfully. In a longitudinal assessment of individuals frequenting a Massachusetts island highly endemic for Lyme disease,[5] untreated patients were identified with relapsing arthritis and fatigue lasting up to 15 years. Importantly, as many as half the individuals identified in that study as infected, based on seroconversion, remained asymptomatic. Subsequent work[6] described untreated patients, symptomatic for an average of 2 years, and emphasized the cognitive difficulties experienced by patients otherwise symptomatic with this chronic infection. This work – and more – illustrates three undisputed facts. First, *B. burgdorferi*, the tick-borne spirochete responsible for Lyme disease, is quite capable of establishing a chronic (ie, many months in duration) infection. Second, this chronic infection, as in many other ongoing inflammatory states, can cause nonspecific symptoms such as malaise, fatigue, and perceived cognitive slowing in addition to more specific clinical manifestations. Third, individuals can be seropositive but asymptomatic following infection.

These observations, combined with misunderstandings about laboratory testing for the diagnosis of this infection, provide the underpinnings of the "debate" about "chronic Lyme disease". Understanding the evolution of this "debate" requires an understanding of the biology of this infection, of the nature of nervous system infection, and of the ways in which nervous system function can be altered by non-neurologic disease.

History and ecology

EM, recognized as a common manifestation of Lyme disease, was first described over a century ago by the Swedish dermatologist, Afzelius,[7,8] who postulated that this was related to the bites of hard-shelled *Ixodes* ticks. Two years after the publication of his observations, two French clinicians[9] published a description of a 58-year-old man who, 3 weeks after a tick bite on the left buttock, developed an enlarging erythroderm at the site of the bite, accompanied by severe sciatic pain. Neuropathic pain subsequently affected both lower and the right upper extremities. Pain persisted for months, and he developed right shoulder weakness. Based on a cerebrospinal fluid (CSF) pleocytosis with elevated protein, and a slightly positive Wasserman test, the authors concluded that he had a non-syphilis spirochetal infection and treated him with neoarsphenamine (preferred treatment at that time for syphilis), and he recovered. This disorder, recognized as including painful radiculitis, lymphocytic meningitis, and subsequently cranial neuritis, came to be known as Garin–Bujadoux–Bannwarth syndrome.[10] European clinicians have been well aware of this tick-bite-associated syndrome for many years, and by the 1950s, were treating it with penicillin.[11]

In the early 1980s, groups in the US[12,13] and Europe[14] established that North American Lyme disease and European EM/Garin–Bujadoux–Bannwarth syndrome were caused by closely related tick-borne spirochetes – *B. burgdorferi* in the US and *Borrelia afzelii* and *Borrelia garinii* in Europe. *B. burgdorferi* is responsible for all Lyme disease acquired in the US. All three strains, as well as several lesser ones

such as *Borrelia spielmanii*, occur in Europe. Most European infections are attributable to *B. garinii*, responsible for the majority of neuroborreliosis, and *B. afzelii*, commonly associated with primarily cutaneous involvement. European and North American borreliosis share many clinical similarities (EM, radiculoneuritis) but have some differences. Once the causative organism was identified, North American Lyme disease became defined as infection with *B. burgdorferi*. Europeans have preferred the terms neuroborreliosis or Lyme borreliosis, referring to the cutaneous manifestations as EM and acrodermatitis chronica atrophicans, or ACA.

Zoonoses such as Lyme disease require specific conditions both to infect humans and to persist in an ecosystem.[15–17] The first requirement is a competent reservoir host, a species that can sustain prolonged, preferably nonlethal, infection. For these *Borrelia* spp., this host consists primarily of field mice (although numerous other small mammals and occasionally birds or even reptiles can serve this purpose). These hosts can maintain a prolonged infection while apparently asymptomatic. While any blood-sucking arthropod could, in theory, ingest spirochete-containing blood, and if it feeds again while spirochetes are still viable, inject spirochetes into another host, the specific interactions of *Borrelia* with *Ixodes* ticks make this the primary, if not sole, competent vector – *Ixodes scapularis* and to a lesser extent *Ixodes pacificus* in the US, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* elsewhere.

Ixodes ticks are born uninfected; there is no transovarial transmission to the egg or larva. Over the course of its typically 2-year life cycle, the tick will ingest a total of three blood meals, one at each life stage. Over the months that follow the larva's ingestion of blood, the tick matures into a nymph, and will then have its second blood meal. The tick will then overwinter, often on a large furry mammal such as a sheep, deer, or bear. Following its final meal on this animal, the adult female tick lays its eggs and dies. Although deer (and corresponding large hosts elsewhere) are often blamed for the transmission of Lyme disease, they are actually only marginally relevant. Without them, the population of ticks will decline. However, if the tick feeds on a deer, this will be its final meal – even if the deer were infected, the tick will never bite another host, so from a Lyme disease perspective, this is a biologic "dead end".

If any of the blood meals contains viable *Borrelia*, these can survive in the tick gut until the tick's next meal. The presence of newly ingested blood at the next meal triggers proliferation of these spirochetes, which then migrate throughout the tick, including reaching its salivary glands. Tick feeding involves days of attachment, during which time tick saliva is injected into the host – injecting anticoagulants, local anesthetics, and other substances required for sustained attachment and feeding. Once the spirochetes migrate to the salivary glands, they can similarly be injected into the host as well. Since the multiplication and migration of *Borrelia* in the tick requires at least 24–48 hours following the initiation of feeding, attachment for periods shorter than this carries very little risk of transmitting infection.[18–20]

Nymphal ticks are the most common cause of human infection. Larvae are uninfected, so even if they were to bite a person, there could be no transmission. Nymphs can be infected and are quite small – about the size of a period on a printed page – so they can be difficult to see. They also substantially outnumber adults – every adult had to be a nymph, but only some nymphs survive to adulthood.

The bite provides the first and best opportunity to interrupt the transmission of Lyme disease. Since the tick must be attached for days to transmit infection, a daily tick check following potential exposure – careful inspection of the skin for attached ticks – with timely removal of any that are found – markedly reduces the risk of infection. Tick removal is best accomplished by insertion of a fine pair of tweezers between the tick mouthparts and the skin, applying slow backward traction. Notably, as the tick feeds, it becomes bloated and engorged. If still tiny and black, it is highly unlikely to have fed sufficiently to have transmitted infection.

Transmission of this infection occurs in many temperate parts of the world where the requisite vectors, infected permissive reservoir hosts, and humans coexist. In the US, it occurs primarily along the east coast, from Maine to Virginia, with small foci of infection in the upper Midwest (Wisconsin, MN) and northern California.[21] The endemic areas have gradually enlarged over the years, but this has been a slow process. In the US, the Centers for Disease Control and Prevention reports about 30,000 cases per year meeting the strict epidemiologic case definition.[21] The number of actual cases probably exceeds that, though by how much is difficult to ascertain.[22]

Laboratory-based diagnosis

In deciding the extent to which a clinical diagnosis of Lyme disease should rely on laboratory confirmation, it is essential first to understand the accuracy of the laboratory techniques, to permit appropriate balancing of clinical vs laboratory data.

Historically, diagnosis of most bacterial infections has relied on in vitro culture and identification of the

responsible organism. This is challenging for some organisms, which are either impossible (*Treponema pallidum*) or difficult (*B. burgdorferi*) to grow in culture. Culture of *B. burgdorferi* requires special medium not generally available in clinical microbiology laboratories. More importantly, other than in EM, the number of organisms present in readily sampled fluids (blood, CSF) appears to be quite low. As a result, even in ideal laboratory circumstances, cultures of CSF obtained from individuals known to have Lyme meningitis are only positive about 10% of the time.[23] Even using the remarkable technical sensitivity of polymerase chain reaction-based techniques does not substantially increase the rate of true positives.

As a result, laboratory support for the diagnosis relies on testing the host immune response to the infecting organism. In most infections, serodiagnosis relies on assessment of acute and convalescent specimens, reflecting the fact that early in any infection, there is little or no measurable antibody, but as infection persists, the host response reflected in the antibody concentration will substantially increase. For reasons probably related to the unfortunate historic comparison to syphilis (where any amount of measurable nonspecific reaginic antibody measured in screening tests is considered to be relevant), Lyme serodiagnosis has often relied on assessment of a single sample. We know that, very early in the course of the illness, such as during the acute rash, as many as 50% of patients will be seronegative.[24] Even during early disseminated infection, occasional patients with Lyme disease-associated facial nerve palsy will only seroconvert weeks after initial clinical presentation.[25] On the other hand, in individuals with symptoms of more than 1- to 2-months duration, essentially every patient is seropositive.[20]

Some studies performed in the 1980s suggested that early but incomplete treatment with antibiotics might permanently abrogate the antibody response.[26] These studies relied in large part on diagnosing patients based on measures of the T-cell response to *B. burgdorferi*. Subsequent work showed this T-cell assay to be quite nonspecific,[20,27] rendering this conclusion incorrect – only if very early treatment eradicates the infection, eliminating any ongoing immune stimulation, would treatment blunt the antibody response. Some have interpreted these early studies as indicating that simply ingesting antibiotics would render a patient seronegative, while the antibiotics were present in the patient's system. There has never been any evidence to support this conclusion, nor is there any biologically plausible basis for making such an assertion.

Consequently, if the data indicate that immunocompetent patients with *B. burgdorferi* infection of more than a few months duration are virtually always seropositive, and if the definition of "chronic Lyme disease" requires symptoms of more than several months duration to be deemed chronic, all patients with "chronic Lyme disease" should be seropositive.

As these conclusions have become more and more firmly rooted in clinical experience, it has become commonplace for the "Lyme literate" to ascribe the symptomatology formerly attributed to "chronic Lyme disease" to chronic infections due to other organisms known to be found occasionally in the same ticks, broadening the definition of "chronic Lyme disease" to include these co-infections.[28] Although laboratory tests confirming the presence of these infections are available, proponents appear either to not rely on the results of the most specific tests or to apply lax interpretive criteria for others, rendering conclusions suspect, or to rely on other tests that have not been subject to rigorous validation. Importantly, there is little if any evidence that these other organisms cause chronic infection, or any of the symptoms attributed to "chronic Lyme disease".

Serologic testing has evolved over the years with most efforts aiming to improve specificity. Initial work used enzyme-linked immunosorbent assays (ELISAs) using sonicated whole organisms as the target antigens; a number of interpretive criteria were chosen to try to balance sensitivity and specificity. In the early 1990s, extensive studies in large populations of patients with and without Lyme disease led to the currently recommended two-tier approach,[29,30] using a highly sensitive ELISA as a screening test, and then a Western blot to provide specificity. It is important to understand that Western blot criteria (Table 1) were not selected based on the uniqueness of any *Borrelia* epitopes but rather on statistical analyses of findings to identify those combinations with the greatest positive and negative predictive values.

Table 1 Western blot interpretation criteria

IgM (two required)	IgG (five required)
24 (OspC)	18
39	21
41 (Fla)	28
	30
	39
	41
	45
	58
	66
	93
For use in acute disease only	For patients with established disease

As a result of these studies, a set of three IgM and ten IgG bands were selected such that individuals with early disease typically have at least two of the three IgM bands, while patients with longstanding disease typically have at least five of the ten IgG bands.[29] Two important facts must be borne in mind. First, the Western blot criteria were developed in individuals with positive or borderline ELISAs. Interpretation in patients with negative ELISAs is quite problematic and should only be attempted with great caution. Second, IgM tests are inherently quite cross-reactive, so false positives are commonplace. Patients with disease of more than 1-month or 2-month duration should be IgG seropositive, so only IgG blots provide reliable information. Any IgM findings in this setting should be considered, at best, uninterpretable, and more correctly as spurious.

Laboratory findings in central nervous system infection

In a significant number of patients with *B. burgdorferi* infection, the spirochete invades the central nervous system (CNS) quite early in the course of the disease.[31] As with any CNS infection, this triggers a local inflammatory response, which can be used to support or refute the conclusion that the CNS is infected. Invasion appears to trigger local production of CXCL13,[32,33] a chemokine that serves to attract circulating B-cells to the site of infection. B-cells that then enter the CNS remain there, producing specific antibody targeting *B. burgdorferi*. Since a small amount of circulating immunoglobulin normally crosses the blood–brain barrier, determining the relative concentrations of *B. burgdorferi*-specific IgG, after normalizing for the relative concentrations of nonspecific IgG, allows for the determination of intrathecal production of specific antibody.[6,34–36] This measure turns out to be highly specific for CNS neuroborreliosis. The major drawback is that the derived index may remain elevated for a decade or more after effective treatment.[37] However, since active infection elicits an inflammatory response, combining CSF serologic information with CSF cell counts and protein concentration provides invaluable diagnostic information. Just as in neurosyphilis, the best measure of resolution of the infection is the normalization of the CSF pleocytosis and elevated protein that are invariably found in active CNS infection.

Clinical phenomenology

Since EM, Garin–Bujadoux–Bannwarth syndrome, and Lyme disease were all clinically defined long before the causative organisms were identified, these disorders were originally described syndromically. Not surprisingly, the replacement of the syndromic definition with diagnosis based on a defined pathophysiology can result in confusion when lab tests and clinical phenomenology do not align. Many assert that the diagnosis of Lyme disease is a "clinical diagnosis" – a statement that is as true for Lyme disease as it is for anything else in medicine. A clinical diagnosis is one made by an informed clinician incorporating all available data. It makes no more sense to ignore relevant laboratory data in a patient clinically suspected to have Lyme disease than it would be to diagnose a lethal brain tumor in a patient with normal brain magnetic resonance imaging (MRI). In any patient, the diagnosis must be deduced based on the balance of the sensitivities and specificities of each of the clinical elements under consideration. In the appropriate context, EM is highly sensitive and specific. In the setting of very early infection, sensitivity of serologic testing is only about 50%, so clinical diagnosis should be based on the rash, not the serology. In patients with Lyme arthritis, sensitivity of serologic testing is for all intents and purposes 100%, so diagnosis requires a positive serology. In patients whose only symptoms are both commonplace and nonspecific – headache, fatigue, and perceived cognitive slowing – it is highly unlikely that even in a highly endemic area would more than 5% of patients with these symptoms have them attributable to this infection. Hence, the specificity of these symptoms is probably no more than 5%. In contrast, in individuals with Lyme disease of more than a month or two duration, sensitivity of serologic testing is over 95%. In this setting, attributing these symptoms to Lyme disease in seronegative patients would be inappropriate.[38]

Cutaneous manifestations

EM, as described by Afzelius, Garin and Bujadoux, and Scrimenti,[39] is almost pathognomonic. Beginning as a small erythroderm at the site of the bite, this gradually expands as spirochetes migrate centrifugally from the initial focus of inoculation. For case definition purposes, it must be at least 5 cm in diameter. Obviously, it will start smaller than this, and if treated rapidly, may not attain this threshold. The erythroderm expands day by day and can become huge – the one described by Garin and Bujadoux involved both buttocks, the abdomen, and thigh of an adult male. The rash can be homogeneous and round but often takes on a target-like appearance as the leading edge becomes erythematous, while more central areas return to their more normal hue. The rash need not be round, its shape dictated by the anatomic areas involved. It is usually surprisingly asymptomatic – not

necessarily pruritic or painful, despite its inflamed appearance. Biopsies generally reveal large numbers of spirochetes, much like in the painless chancre of syphilis. However, the clinical appearance and evolution are so typical that biopsy is rarely needed. The frequency with which EM occurs has been debated. In children, who presumably have parents attentively inspecting them at bath time, about 90% of infected children develop EM.[40] In adults, where the rash might occur in areas not easily or routinely inspected, estimates are generally in the range of a half to two-thirds.

Spirochetes can disseminate hematogenously from this original nidus. In the US, about 25% or more of patients will develop multifocal EM as a result of this,[41] each of the secondary EMs evolving in a manner similar to the original one. This only occurs in about 5% of European patients. In contrast, European patients may develop a *Borrelia* lymphocytoma – a dense lymphocytic infiltrate of the earlobe or areola of the breast, something seen very rarely in the US. Europeans with chronic untreated infection can also develop acrodermatitis atrophicans – a tissue paper-like thinning with purplish discoloration of the skin of a leg. This has never been reported in a US patient.

Non-neurologic extracutaneous symptoms

Early descriptions of Lyme disease included the occurrence of otherwise unexplained heart block in about 5% of patients. For unclear reasons, this has been less evident in more recent series. It has been described occasionally in European patients, but the incidence is certainly no more than a single-digit percent of infected individuals.

The original defining phenomenology in the US patients was Lyme arthritis.[3] This is a relapsing remitting large-joint oligoarthritis – affecting a knee, elbow, shoulder, or hip. Typically, one joint is involved at a time, spontaneously becoming red, painful, and swollen, and then resolving after a few weeks. At different times, different joints can be involved. Small joints such as fingers, toes, or spine facets are involved infrequently. Arthritis too seems to be becoming less frequent with increased early recognition and treatment of Lyme disease.

Neuroborreliosis

The range of different disorders considered to be manifestations of nervous system involvement with *B. burgdorferi*, *B. afzelii*, and *B. garinii* can be considered to fall into three distinct groups.[42] The "classic triad" occurs in early acute infection, presenting as acute inflammation in one or a few nerves or of the meninges.[4,9,43] Occasional patients will have infection and inflammation involving the spinal cord or brain. Patients with more indolent, longstanding infection may have subacute-to-chronic infection of the peripheral nervous system (PNS)[44] or CNS.[6] Other patients – with evidence of active infection outside the nervous system – may have what has been termed "Lyme encephalopathy"[45,46] – symptoms of cognitive and memory difficulty, fatigue, and malaise, identical to those seen in patients with other active infectious or inflammatory states.

"Classic triad"

Acute nervous system involvement occurs in 10%–15% of infected individuals – in both Europe and the US[47] – and takes quite similar forms in both regions. The classic triad includes lymphocytic meningitis and painful radiculoneuritis, as described years ago by Garin and Bujadoux, as well as cranial neuritis.

Radiculitis, a term used generally to describe inflammation of spinal nerve roots, causes sciatica-like neuropathic pain, typically involving one or a few dermatomes, most commonly in a limb but occasionally the trunk. Patients often will have weakness or diminished deep tendon reflexes in muscles innervated by the affected dermatome. Non-Lyme disease radiculopathy is almost always caused by mechanical compression; patients with neuroborreliosis can develop remarkably similar symptoms, attributed to inflammation of the symptomatic nerve root or roots. Precisely the syndrome described by Garin and Bujadoux in 1922, it is now clear that this is just one clinical presentation of PNS involvement in Lyme disease.[44] It appears likely that these clinically varied manifestations are all various forms of what is known as a mononeuropathy multiplex, a common type of peripheral nerve involvement in inflammatory diseases (vasculitides, infections such as leprosy) or other vasculopathic disorders, such as diabetes mellitus.

Although all rooted in the same pathophysiologic process, clinical manifestations of PNS Lyme will vary with the nerve(s) involved. If a single nerve is involved, the patient may develop a mononeuropathy. A single nerve root can produce signs and symptoms indistinguishable from a mechanical radiculopathy associated with disk herniation – with severe radicular neuropathic pain and segmental muscle denervation and reflex loss. Other patients may develop a plexopathy, involving either the brachial or lumbosacral plexus.

Cranial nerve involvement is also quite common, with the facial nerve, either unilaterally or occasionally bilaterally,

involved in the majority of patients with Lyme disease-associated cranial neuropathies. It is important to emphasize that facial palsy is not a subtle finding – patients have severe drooping of the side of the face, and are typically unable to seal the lips, close the eye, or wrinkle the forehead on one side. Recovery is to be expected in this disorder, but the initial presentation is typically quite dramatic – and distressing to the patient. Other cranial nerves can be involved – the trigeminal nerve causing numbness and pain, the nerves to the extraocular muscles causing paralysis of innervated eye muscles and diplopia, or the acousticovestibular nerve affecting hearing and balance. These are all distinctly uncommon; involvement of the optic or olfactory nerves occurs rarely if ever.[48] Similarly involvement of cranial nerves 9–12 occurs very infrequently.[49] As with peripheral nerve involvement, facial palsy and these other cranial neuropathies appear to be differing manifestations of the same process – a mononeuropathy multiplex.

Each of these disorders – cranial neuritis, radiculoneuritis, and lymphocytic meningitis – can be considered typical of nervous system Lyme disease. It is therefore worth considering the specificity of these disorders for the diagnosis. In an observational study from an area highly endemic for Lyme disease, approximately 25% of adult cases of facial nerve palsy were attributable to Lyme disease.[25] Cross-sectional studies of patients with "aseptic meningitis"[50–53] suggest that in summer months, in areas highly endemic for Lyme disease, between one in two and one in five patients with lymphocytic meningitis might have this infection. Lumbar radiculopathy is estimated to affect 3%–5% of the adult population;[54] cervical radiculopathy is slightly less prevalent. Since radiculopathy affects no more than 5% of the 30,000 patients with Lyme disease reported to the Centers for Disease Control and Prevention annually, this would translate to 1,500 cases per year, a very small fraction of the number of mechanically caused cases. From this, it should be clear that the specificity of even these very specific neurologic manifestations of Lyme disease is quite low – no more than 25% for facial nerve palsy, perhaps one in three for lymphocytic meningitis, and much less than 1% for radiculopathy – in areas highly endemic for Lyme disease. In areas where the incidence of Lyme disease is much lower, the potential positive predictive value for one of these presentations for the diagnosis of Lyme disease would drop precipitously. Even in endemic areas, a finding with no better than a 50:50 chance of predicting the diagnosis would never be considered a useful basis for making a clinical diagnosis.

Patients with more longstanding and indolent infection may have less acute forms of nervous system involvement. Originally described in European patients with acrodermatitis,[55] there may be more diffuse, widespread involvement of peripheral nerves, clinically mimicking the much more common stocking–glove type of peripheral neuropathy. A very similar disorder was described in patients with chronic, untreated Lyme arthritis.[56,57] In both, more detailed analysis suggests that this too is pathophysiologically a form of mononeuropathy – in this case, what is referred to as a confluent mononeuropathy multiplex – with mild diffuse involvement of multiple small nerves.

Lyme encephalitis vs Lyme encephalopathy

The issue that has caused the greatest confusion about nervous system Lyme disease relates to possible CNS involvement. Other than lymphocytic meningitis – a disorder that may cause severe headache but does not damage the brain itself – parenchymal CNS infection is remarkably rare. In European patients with Lyme radiculitis, the nerve root inflammation may extend proximally into the adjacent spinal cord, causing myelopathic changes – a disorder reported only anecdotally in the US (unpublished observations). Brain involvement was described primarily in the European literature in the 1980s.[58,59] More recent reports are extraordinarily difficult to find, perhaps reflecting more aggressive early recognition and treatment of this infection. Similarly, in some rare cases, patients with apparent parenchymal brain involvement have been reported from the US,[6,57] but this too is now remarkably infrequent. What little evidence exists about these patients indicates that it is due to active brain infection. Imaging studies appear inflammatory, PET scans appear hypermetabolic,[60] CSF is inflammatory, and most patients have evidence of localized production of specific anti-*Borrelia* antibody within the CNS.[6,61,62]

In contrast to these rare occurrences in patients, in the days when patients with longstanding Lyme arthritis were frequently seen, it was almost the norm for them to describe cognitive slowing, fatigue, and memory problems. With extensive testing, including brain MRI, CSF examinations, neurophysiologic testing, and more, virtually none had evidence of CNS inflammation or infection.[6] Some work suggested that this might be related to elevated concentrations of cytokines or other potential neuromodulators[63,64] produced in the periphery and then entering the brain, but the mechanism remains unclear. What is clear is that the clinical phenomenology is indistinguishable from the "toxic metabolic" encephalopathy

seen in active rheumatoid arthritis, sepsis, other serious infections, or even in patients receiving therapeutic interferon. It is equally clear that in virtually none of these individuals is there any evidence of CNS infection with *B. burgdorferi*, or of any potentially related inflammatory state. Rather, this seems to be the ubiquitous encephalopathy or delirium seen in innumerable medical patients with active systemic infection or inflammation. Just like that state, this alteration of cognitive function is reversible when the infection or inflammation resolves (although often not immediately) and does not require any specific alteration of treatment. Specifically, this does not require the selection of antimicrobial regimens based on their ability to penetrate the blood–brain barrier.

Treatment

The *Borrelia* spp. responsible for Lyme disease remain highly sensitive to readily available antibiotics, both in vivo and in vitro.[20] Early studies in patients infected for extended periods of time demonstrated microbiologic cures in the vast majority of such individuals.[20,65] Patients with significant end organ damage – those with severe arthritis or with longstanding brain or spinal cord inflammation – might have some residua from already established damage, but progressive or recurrent disease is distinctly uncommon. Although meningeal dose penicillin and ceftriaxone were both introduced to assure adequate treatment of CNS infection, there are now multiple studies demonstrating that oral doxycycline attains sufficient concentrations in the CSF to be as effective as intravenously administered ceftriaxone, at least in European patients[66,67] (Table 2).

Table 2 Commonly used antimicrobial regimens

Disorder	Regimen
Non-neurologic disease	Amoxicillin 500 mg po tid or doxycycline 100 mg po bid or cefuroxime axetil 500 mg po bid, all for 14–28 days
Acute neuroborreliosis (meningitis, radiculitis, cranial neuritis)	Ceftriaxone 2 g/day for 14–21 days or cefotaxime 2 g tid for 14–21 days, or penicillin 24 MU/day ×14–21 days or probably po doxycycline 200–400 mg/day for 21–42 days
Encephalomyelitis	Ceftriaxone 2 g/day for 14–28 days or cefotaxime 2 g tid for 14–28 days, or penicillin 24 MU/day ×14–28 days
Chronic or recurrent neuroborreliosis (eg, treatment failure after first course)	Ceftriaxone 2 g/day for 28 days or cefotaxime 2 g tid for 28 days
Disease resistant to oral treatment	Ceftriaxone 2 g/day for 14–28 days or cefotaxime 2 g tid for 14–28 days, or penicillin 24 MU/day ×14–28 days

Note: Tetracyclines such as doxycycline should not be used in pregnant women or in children aged 8 years or under.
Abbreviations: po, oral; tid, thrice daily; bid, twice daily.

In contrast to the overwhelming evidence that *Borrelia* infections are readily cured with antimicrobial therapy, there is abundant evidence that antibiotic treatment is ineffective in patients with persistent fatigue and cognitive symptoms following appropriately diagnosed and treated Lyme disease.[68–71]

Conclusion

Lyme disease, defined as infection with *B. burgdorferi* or the two closely related European spirochete pathogens *B. afzelii* and *B. garinii*, infects the nervous system in up to 15% of patients. Untreated infection, particularly with joint involvement, can be chronic, lasting years. That said, it is essential to understand that the fatigue and cognitive difficulty seen in many individuals with Lyme disease are neither caused by or evidence of nervous system infection nor in any way specific to this disease.[72] Patients without Lyme disease may develop the same disabling symptoms including severe fatigue and cognitive difficulty, a disorder formerly referred to as chronic fatigue syndrome, and for which the label "systemic exertion intolerance disease" has recently been suggested.[1] Although the latter is both real and disabling, and appears to occur following any number of infections – including possibly Lyme disease – there is no evidence that it is caused by persisting infection with *B. burgdorferi*, other tick-borne pathogens, or any other as yet identified pathogen. As such, treatment of this symptom complex with antibiotics is unlikely to be helpful to patients but does incur substantial risk. This, combined with the impact of excessive antibiotic usage on the development of widespread antibiotic resistance among more potentially lethal pathogens, and the significant health care resource utilization and cost associated with prolonged administration of parenteral antibiotics, makes such treatment ill advised.

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Expert witness, defending physicians in medical malpractice cases in which they have been accused of failure to diagnose or treat Lyme disease. The author owns equity in several pharmaceutical companies none of which is relevant to this topic. He has also received royalties from UpToDate and for "Lyme Disease, an evidence based approach" published by CABI in 2011. The author reports no other conflicts of interest in this work.

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Misconceptions about Lyme Disease: Confusions Hiding behind Ill-Chosen Terminology

"The beginning of wisdom is to call things by their right names."

—Ancient Chinese proverb

Nomenclature influences perceptions of reality and frames ensuing discussions. Imprecision contributes to misinterpretation of observations and studies, altering clinicians' approaches. The impact of imprecision and novel reinterpretation of terminology can be seen in the Lyme disease debate. A quarter century after its initial description, a review of the terminology contributing to confusion about Lyme disease is needed.

Lyme disease is treatable and curable with antibiotics (1–4), especially if treated promptly, usually with an excellent long-term prognosis. The term "promptly" taken out of context suggests one *must* treat without any delay. In fact, even untreated patients have a good prognosis. A 10- to 20-year follow-up of patients at Yale's Lyme Disease Clinic from 1976 to 1983, many of whom were not treated for early Lyme disease, shows that the patients with erythema migrans did not differ from normal controls in current symptoms, physical findings, results of neuropsychological testing, or responses to the Short-Form 36 Health Assessment Questionnaire (5). However, significant long-term sequelae occurred in patients with untreated facial palsy who probably had disseminated Lyme disease at initial evaluation and probably required intravenous therapy (5).

"Chronic Lyme Disease"—A Term in Search of Definition

Despite this generally optimistic picture, claims of persisting infection and antibiotic unresponsiveness have contributed to anxiety. "Chronic Lyme disease" (6, 7) is a common clinical diagnosis in some geographic areas (8, 9) and is based on thinking that is at odds with scientifically validated findings. No objective physical findings or unique historical features define "chronic Lyme disease," a term used by support groups and their few physician allies, not the academic medical community. Although the subject of much debate, "chronic Lyme disease" is not well defined. The term is usually applied to patients with symptoms, such as fatigue, achiness, malaise, and difficulty with concentration and memory, after treatment of documented Lyme disease or illnesses thought to be Lyme disease, or in patients without preceding illness (9). Once the term Lyme disease is applied, it *is* Lyme disease, forever and irrefutably, a diagnosis often "reaffirmed" by cross-referral between "Lyme literate" physicians.

Many patients receive repeated courses of antibiotics, with transient or waning responses, leading to more or a combination of antibiotics. Occasionally, this course of treatment can go on for years, with little relief. Originally, "chronic" was used in the context of Lyme arthritis, which, in the era before it was determined that Lyme disease was responsive to antibiotics, persisted for years, finally resolving spontaneously (10). Antibiotics are of proven value for Lyme arthritis (11); treatment of early Lyme disease usually prevents arthritis.

A Nexis-Lexis review reveals that "chronic" was first applied to Lyme disease in 1985: Lyme disease was "a potentially chronic and debilitating illness transmitted by tick bites" (12). In 1986, "chronic" referred to outcomes if antibiotics were not administered (13). A letter to the editor by Drs. Falvo and Nadelman urged more support for research: Lyme disease could cause "birth defects, fetal death, unilateral blindness, and chronic debilitating arthritis" (14) (the first and second occurrences still unproven, the third rare, and the last well reported). An adjective appropriate for Lyme arthritis before the identification of antibiotic responsiveness (1, 2, 10, 11) was used to describe patients not responding to antibiotics, suggesting that antibiotics do not kill *Borellia burgdorferi.*

Some Internet sites, support and advocacy groups, and some clinicians claim that the truth is deliberately being obscured: that "chronic Lyme disease" is far more common than the authorities allow us to know; antibiotics are often not curative; infection can be controlled only by long-term antibiotic therapy, often more than insurance companies allow; serologic tests are inaccurate and often yield falsely negative results, thereby incorrectly discouraging diagnosis; the prognosis is not nearly so rosy as "they" (the nefarious academic experts) claim;

Table 1. **What Is Accepted about Lyme Disease**

Lyme disease is also known as Lyme borreliosis and occasionally as erythema migrans disease.
Lyme disease is a multisystem inflammatory condition of the temperate Northern hemisphere caused by spirochetes collectively known as *Borrelia burgdorferi* sensu lato.
Borrelia burgdorferi sensu lato consists of three pathogenic genospecies: in the United States, Lyme disease is caused by *B. burgdorferi* sensu stricto; in Europe and Asia, Lyme borreliosis is due to infection with *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto.
Lyme disease is spread by *Ixodes* ticks: *I. scapularis* in the northeastern, north midwestern, and Middle Atlantic states of the United States and *I. pacificus* along the northern Pacific coast; *I. ricinus* in Europe; and *I. persulcatus* in Asia. (*I. scapularis* is also found in the southeastern United States, but little if any Lyme disease is reported from that region.)
Most reported cases of Lyme disease in the United States are from southern New England, the Middle Atlantic states, Wisconsin, Minnesota, and northern California. Scattered cases have been reported from the upper South and the Midwest. Erythema migrans–like lesions have been reported from other regions, such as North Carolina (15) and Missouri (16), without serologic confirmation of exposure to *B. burgdorferi*.
Lyme arthritis was described in studies of an outbreak of presumed juvenile rheumatoid arthritis in Connecticut; the association with preceding erythema migrans (then known as erythema chronicum migrans) and tick bites became apparent soon thereafter.
As nonarticular features were identified, the spectrum of Lyme disease became clear and the similarities of Lyme disease with clinical findings from erythema migrans in Europe emerged.
After identification and cultivation of pathogenic borrelial genospecies from tick and human specimens, serologic tests measuring anti–*B. burgdorferi* antibodies were developed and criteria for their interpretation were established.
The clinical spectrum of Lyme disease includes effects on the skin, heart, peripheral and central nervous systems, and the musculoarticular system; these effects have been reviewed elsewhere (1, 2, 5, 7, 10, 17).
Lyme disease has been described using three phases of infection: 1. Early localized disease: erythema migrans and associated symptoms 2. Early disseminated disease: multiple erythema migrans and associated symptoms; Lyme carditis; neurologic features, including facial (and other cranial nerve) palsies, lymphocytic meningitis, and radiculoneuropathies 3. Late disease: neurologic features, including peripheral neuropathies and chronic mild encephalopathy; arthritis, including migratory polyarthritis and/or monoarthritis
The pathogenesis of Lyme disease is not entirely understood, but some of the features of Lyme disease depend on the presence of the organism at the site of damage. Immunologic mechanisms, summarized elsewhere (18), may underlie other features of the disease.

many lives have been ruined; and many people have died (8–10). None of these claims is supported by scientific medical literature, yet they disseminate regularly, acquiring verity by their repetition.

By focusing on terminology, we may understand how some confusion has been promulgated and exacerbated. Insight may aid in clarification and be useful in addressing non–Lyme disease areas of contention. The contents of **Table 1** are probably acceptable to most researchers and clinicians who think about Lyme disease. Beyond these "absolute" facts lie concepts involving terms such as "very unlikely," "has been reported," "usually," or "in most patients"—modifiers describing "shades of gray." Physician-scientists are good at communicating facts, but "shades of gray" are often difficult to convey; the more precise one tries to be about the limits of our knowledge, the more doubts are planted, and the more misinterpretations occur. This is the root of endless debate, the home of a Cartesian dualism of sorts.

RATIONALISTS VERSUS EMPIRICISTS

The opposing sides in this debate about the true nature of Lyme disease can be described as "rationalists" and "empiricists." Rationalists use scientific studies, both clinical and molecular, to develop models of disease and appropriate diagnostic and therapeutic responses. Empiricists base models on community events, developing diagnostic and management schemas that are compatible with observations, but often at odds with scientific conclusions. In conveying their message, empiricists often adopt terminology that contradicts the terminology's intended meaning. Most published clinical and basic research on Lyme disease is from rationalists, physicians searching for objective evidence of infection. The empiricists' ranks include support groups and physicians in practices devoted to the care of patients with "chronic Lyme disease," who are given a diagnosis and are treated on the basis of nonspecific symptoms, such as fatigue, cognitive dysfunction, and pain, rather than objective evidence of infection. Empiricists "listen to the patient" rather than follow the advice of scientific studies, as if these were mutually exclusive. Rationalists fear that physicians, with the help of misinterpreted test results, occasionally misdiagnose serious illnesses as "chronic Lyme disease." Empiricists often diagnose without formulating a differential diagnosis—this *is* Lyme disease. Some call these two opposing views "two schools of thought," but I prefer to call them proponents of "reality" and "alternative reality." The sage of Baltimore, H.L. Mencken, could have been referring to this divide when he penned his introduction to the first American edition of *The Antichrist* by Nietzsche: "The majority of men prefer delusion to truth. It is easier to

grasp. Above all, it fits more snugly into a universe of false appearances . . . ."

"Delusions" may satisfy needs; facts offer cold comfort to the sufferer. When false appearances assume the cloak of "reality," "alternative reality" is established.

The debate between these two groups includes diagnosing the illness, use of testing in diagnosis and management, duration and forms of therapy, prognosis, and defining a cure. Inattention to details and facts, their manipulation, and incorrect citation have fed this occasionally rancorous disputation (12), further confusing most clinicians and patients on the sidelines and causing the suffering of innocent patients and families.

Lyme Disease as "The Great Imitator"

The term "The Great Imitator" as applied to Lyme disease (an attempt to form an analogy with another spirochetal disease, syphilis [19]) contributed to confusion. The comparison was meant not to denote clinical similarities between these diseases but to suggest that, as with syphilis in a previous era, Lyme disease included a broad range of findings and mimicked other diseases. However, it soon became clear that most cases of Lyme disease are recognizable in a well-described spectrum (20–25), the rare exceptions being, by definition, outliers (26). Most patients have objective abnormalities (2). Used correctly, testing is helpful: Immunologic (antibodies in serum and cerebrospinal and synovial fluids) (27), molecular biological (polymerase chain reaction identification of specific DNA), electrophysiologic (heart and neurologic), and neuropsychological (28) tests can support the diagnosis (8). Instead, "The Great Imitator" was misinterpreted as suggesting that Lyme disease routinely mimics and is mimicked by many other diseases. Some empiricists believed Lyme disease was difficult to explicitly diagnose and had to be part of the differential diagnosis of *all* problems of *all* diseases it *might* imitate (11). Lyme disease is often considered in many patients whose symptoms do not explicitly suggest Lyme disease and who receive that diagnosis merely because no other diseases can be explicitly diagnosed.

Centers for Disease Control and Prevention Criteria: Use and Misuse

The belief that Lyme disease is often overlooked is expressed as dissatisfaction with (even anger at) the Centers for Disease Control and Prevention (CDC) surveillance criteria as dangerous stricture, inexplicably designed to minimize reports of "accepted" cases (29). The criteria were designed for surveillance (and are useful as entry criteria for studies) but were not meant for diagnostic purposes. Tabulation of cases that satisfy criteria allows comparison from year to year, assessing numeric and geographic expansion. Not all cases meet the criteria (30).

"Lyme Disease Is a Clinical Diagnosis"

The original meaning of "Lyme disease is a clinical diagnosis" was that one should not diagnose solely on the basis of test results but also on historical and physical evidence that explicitly suggests Lyme disease. Such findings should suggest *the possibility* of Lyme disease—no finding, even in endemic areas, is diagnostic. The phrase has been manipulated into something far from its original intent. History and physical examination may not suggest Lyme disease, serologic testing may yield negative results, but one makes a "clinical diagnosis" simply because one decides the nonspecific symptoms (for example, fatigue and achiness) are due to Lyme disease: The "patient had symptoms compatible with Lyme disease" and lived in an endemic area. Lyme disease becomes a "diagnosis of exclusion" (9), often without any effort to exclude other diagnoses. "Virus-like" symptoms, such as fever, myalgia, and arthralgia, are common in early Lyme disease, although respiratory and gastrointestinal symptoms are uncommon. Nonetheless, "flu-like" symptoms are diagnosed as Lyme disease and are another example of imprecision; patients with acute viral syndromes years into the course of long-standing clinical problems are said to have the "flu-like" symptoms of Lyme disease.

"Symptoms Compatible with Lyme Disease" and the Misuse of Serologic Testing

A patient with "symptoms compatible with Lyme disease," absent physical findings, may receive a diagnosis of Lyme disease because of positive results on "Lyme disease tests" or "Lyme serologies." These tests measure antibodies binding *B. burgdorferi* in vitro, nothing more. The antibodies *may* be a marker of exposure, but they do not document current infection and may indicate a false-positive result. The intrinsic degeneracy of the humoral immune response assures that antibodies against other organisms may bind in such tests. Thus, a positive

result on serology does not prove *B. burgdorferi* exposure. Bayesian theory predicts the clinical utility of testing—minimal positive predictive value if a priori likelihood was low (9). Serologic tests were developed as an adjunct to clinical diagnosis (31), and a positive test result increases a priori likelihood. However, a weakly positive test result is often the sole "evidence" favoring Lyme disease. "Lyme disease test" and "Lyme serologies" are misleading terms, suggesting the incorrect but seemingly logical conclusion that a positive result diagnoses Lyme disease. There is no such thing as a "Lyme disease test" (8, 9, 31).

The second test in the two-tiered serologic approach is immunoblot. Antibodies to individual proteins ("bands") are assigned an approximate molecular mass in kilodaltons. The CDC recommendation is that positive or equivocal results on enzyme-linked immunosorbent assays be supplemented by immunoblot (31) because the latter is more specific and the former, a first-level test, is intended to be more sensitive. Criteria were established for interpretation of results on immunoblot—IgM assays for early disease and IgG for later disease (31).

The "clinical diagnosis" of Lyme disease is often incorrectly secured by positive serologic results with negative findings on immunoblot (a "biologic false-positive" test result, borrowing again from syphilis). If a priori belief in the diagnosis is sufficient, a negative test result is dismissed—after all, "we all know how inaccurate the tests are." Regardless of results, the "clinical diagnosis" stands. Serologies are useful; their major limitation is the knowledge of the clinician who orders them and interprets the results.

Misinterpretation of immunoblot was common with earlier assays, often because of the assignment of "positive" or "negative" results to each "band," with the "positive" band being misread as a positive immunoblot finding. New reportage suggests referring to small print at the bottom of the report and understanding that IgM and IgG criteria should be used for early and later infection, respectively. Isolated IgM reactivity does not indicate chronic Lyme disease—IgG reactivity should have emerged. IgM reactivity occurs in *early* infection but has been misinterpreted as indicating *active* infection. Seroreactivity, even with IgM, can persist long after cure—persisting seroreactivity is not evidence of ongoing infection (27, 32–34). Nonetheless, a single reactive band has been misinterpreted as indicating infection, and "chronic Lyme disease" has been misdiagnosed because of seroreactivity persisting after therapy.

The Permanence of a Lyme Disease Diagnosis

Even if proof of diagnosis at inception is tenuous, subsequent physicians may accept the previous Lyme disease diagnosis, often without independent scrutiny, as if it were proven beyond doubt. The diagnosis becomes permanent (35), all future findings perforce Lyme disease-related, making post hoc ergo propter hoc ("after this, therefore, because of this") logic all the more fallacious since the initial diagnosis was incorrect. The neologism "chronic Lyme disease" is the most damaging term in this developing imprecise lexicon. The diagnosis becomes life-long, a misdiagnosis causing missed diagnoses; the explanation for the patient's problems is never identified, and the accepted misdiagnosis prevents further search. Musculoskeletal pain is "Lyme arthritis,"and cognitive dysfunction is "central nervous system disease."

Many patients cleave to "chronic Lyme disease" despite lack of response, expense, and significant toxicities (36). It is human nature to seek explanations. The fear of the unknown can be greater than the fear of even incurable chronic disease. Anxiety and fear drive the pursuit of diagnosis, testing, and treatment (37). Achieving a diagnosis, even one of incurable "chronic Lyme disease," may offer patients with chronic symptoms comfort and assurance.

The Effects of Antibiotics on "Chronic Lyme Disease"

Symptoms that develop or worsen during antibiotic therapy are "Herxheimer-like" reactions. A Jarisch–Herxheimer reaction occurs in about 10% of patients within days of initial antibiotic administration and not after subsequent courses. Worsening of symptoms without objective findings with a periodicity of about 28 days (the organism's "natural rhythm") is neither a Jarisch–Herxheimer nor a Herxheimer-like reaction; neither Jarisch nor Herxheimer would recognize these as what they described (36).

If symptoms persist despite antibiotic use, there may be ongoing infection (36) requiring further treatment. This could include months or years of oral or intrave-

Table 2. **Lyme Disease Terminology: Present and Proposed**

Current Term	Proposed Substitution	Why the Change Is Needed
Clinical diagnosis	No change	It is and always will be a clinical diagnosis, but the diagnosis must be based on explicit evidence of the disease and never made as a "diagnosis of exclusion."
Diagnostic tests	Sero-confirmatory tests	Testing should never be used as the sole basis for the diagnosis of Lyme disease. A positive test result is not diagnostic; it merely increases the likelihood of the diagnosis previously based on explicit clinical evidence. "Vide supra"–Bayes theorem: if the clinical suspicion of Lyme disease is low, a positive test result does not make the diagnosis. In a case with a high a priori likelihood of Lyme disease, a positive test result can do no more than confirm the clinician's conclusion of a reasonably high likelihood of disease. Some argue persuasively that even "sero-confirmatory" is too strong a term—perhaps more accurate (but less euphonious) would be "sero-suggestive."
Lyme disease test	Anti–*Borrelia burgdorferi* antibody test	The test does not diagnose Lyme disease. The test merely identifies antibodies binding to *Borrelia burgdorferi* in vitro, antibodies possibly not made in an immune response to *B. burgdorferi* in the first place. So, why not call it what it really is?
Flu-like symptoms	Viral syndrome	Influenza often includes prominent pulmonary symptoms that are relatively rare in Lyme disease. Likewise, gastrointestinal symptoms are not prominent in early Lyme disease.
Chronic Lyme disease	–*	This term is of no proven value in the management of patients with established Lyme disease. Until scientific studies prove that chronic *B. burgdorferi* infection exists, especially following what would otherwise seem to be adequate antibiotic therapy, this term should not be used. This is in contrast to the term "post–Lyme disease syndrome," which appears to describe a real clinical entity not associated with ongoing *B. burgdorferi* infection.
Lyme disease	Lyme borreliosis	The term "Lyme disease" means so many different things to different people at this point that a new name emphasizing the underlying infection with *B. burgdorferi* might help shift the focus back where it belongs—to a multisystem inflammatory disease due to an infection with *B. burgdorferi*.
Symptoms of Lyme disease	–*	The symptoms seen in patients with Lyme disease are not unique for any of the clinical manifestations of the diseases, but most emphatically for early disease. Patients with early Lyme disease may have fever, myalgias, and arthralgias, suggesting a "viral syndrome," but such symptoms are not unique to or specific for this infection; such symptoms in the summer or early fall are probably related to a viral infection. Thus this term is rendered meaningless by its lack of specificity.
Symptoms compatible with Lyme disease	–*	This term is also rendered useless by its imprecision and bias. So many of the symptoms of Lyme disease are found in other diseases that there is no way to directly ascribe them to Lyme disease—nearly all nonspecific symptoms thereby are "compatible" with Lyme disease. "Symptoms compatible with Lyme disease" takes in the entire spectrum of medicine and is a needlessly biased and suggestive term that should be abandoned.
"Lyme literate"	–*	A neologism, coined by lay support groups, that seems to identify clinicians subscribing to the empiricists' approach. "Listening to the patient" is not an attribute unique to clinicians in this group, just as all "Lyme literate" practitioners do not eschew the development of an appropriate and thorough differential diagnosis in order to make Lyme disease a "diagnosis of exclusion."

* The absence of a proposed replacement suggests that this term should be deleted from use for the reasons noted.

nous antibiotics, or combination antibiotics, occasionally including agents that are inactive against *B. burgdorferi* (for example, atovaquone). With few exceptions, such as *Tropheryma whippelii* and mycobacteria, no bacteria necessitate long-term antibiotic therapy as is used for "chronic Lyme disease."

Long-term antibiotic therapy, however, is needed because *B. burgdorferi* becomes dormant or hides within cells (38–41); these interactions protect the organism from antibiotics (42) (in vitro phenomena not documented in vivo) so that organisms are not responsive. Empiricists call proven regimens "conservative therapy" (1–3, 20, 22), a pejorative term suggesting incompetence of rationalist approaches. Transient response indicates a need for more treatment; many antibiotics have nonantimicrobial effects (43). Inadequacy of treatment is the only explanation for lack of response; the nonexistence of infection is not considered. A recent National Institutes of Health–funded trial showed that 3 months of antibiotics for "chronic Lyme disease" was ineffective (44); unfortunately, empiricist criticism will probably dismiss the results.

Peer-reviewed experience from academic referral centers indicates that most patients unresponsive to antibiotics do not have Lyme disease (35, 45–48). Some never had it; others were cured of *B. burgdorferi* infection. Symptoms following Lyme disease should not necessarily be ascribed to preceding *B. burgdorferi* infection. Such patients should be evaluated for objective evidence of inflammation and organ damage and evidence of cur-

rent infection; the post hoc ergo propter hoc approach has often proven hazardous. There are "post-Lyme disease syndromes," such as fibromyalgia and depression, that do not respond to antibiotics (35, 44–47); fibromyalgia following Lyme disease is not due to ongoing infection. Objective neurologic deficits may be due to irreversible brain damage from Lyme disease, but if previous therapy was adequate, antibiotics are unlikely to be useful. Anxiety elicited by fears of incurable "chronic Lyme disease" does not respond to antibiotics. Above all, one must individualize the approach to the patient.

The Effects of "Chronic Lyme Disease" on the Patient

Long-term antibiotic therapy is not without financial and physical costs, such as bone marrow toxicity, central line sepsis (49, 50), or even death (51). Hidden in this epidemic of chronic disease and debility is the psychological cost of accepting a disease as a permanent part of yourself, that you will never be cured, that the disease will be with you forever, no matter how powerful the drugs or how aggressively they are given. Assumption of the "sick role" leaves an invisible scar that may be the most devastating effect of "chronic Lyme disease" (8).

Proposals for Changes in Terminology—Reducing Bias in the Language

Twenty-five years after the description of Lyme disease we have come far: clinical features are well described, accurate tests support the diagnosis, effective therapy is available, and there is an effective vaccine. It is time to reflect on the jargon that contributes to misdiagnosis and mistreatment. Table 2 highlights some of the more troublesome terms and proposed substitutions. Use of less suggestive, accurate, unbiased terminology will help us focus on Lyme disease and take proper care of our patients, many of whom have been ill-served by cant and rhetoric. Those convinced that "chronic Lyme disease" is real, that long-term antibiotics are needed, will not be easily deterred by this analysis. However, the unbiased may use this analysis to carefully inspect the rationalist and empiricist approaches and decide which fulfills our sacred responsibility to "do no harm." Issues considered in this review may help physicians deal rationally with future clinical controversies.

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CLINICAL RESEARCH STUDY

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Chronic Coinfections in Patients Diagnosed with Chronic Lyme Disease: A Systematic Review

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ABSTRACT

PURPOSE: Often, the controversial diagnosis of chronic Lyme disease is given to patients with prolonged, medically unexplained physical symptoms. Many such patients also are treated for chronic coinfections with *Babesia*, *Anaplasma*, or *Bartonella* in the absence of typical presentations, objective clinical findings, or laboratory confirmation of active infection. We have undertaken a systematic review of the literature to evaluate several aspects of this practice.

METHODS: Five systematic literature searches were performed using Boolean operators and the PubMed search engine.

RESULTS: The literature searches did not demonstrate convincing evidence of: 1) chronic anaplasmosis infection; 2) treatment-responsive symptomatic chronic babesiosis in immunocompetent persons in the absence of fever, laboratory abnormalities, and detectable parasitemia; 3) either geographically widespread or treatment-responsive symptomatic chronic infection with *Babesia duncani* in the absence of fever, laboratory abnormalities, and detectable parasitemia; 4) tick-borne transmission of *Bartonella* species; or 5) simultaneous Lyme disease and *Bartonella* infection.

CONCLUSIONS: The medical literature does not support the diagnosis of chronic, atypical tick-borne coinfections in patients with chronic, nonspecific illnesses.

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KEYWORDS: Anaplasma; Babesia; Bartonella; Borrelia burgdorferi; Coinfection; Lyme disease

Lyme disease is the most commonly reported vector-borne infection in the US with over 30,000 confirmed or probable cases in 2011.[1] Lyme disease is caused by infection with the spirochete *Borrelia burgdorferi* and transmitted by *Ixodes* spp. ticks.

While many aspects of Lyme disease are well accepted by the mainstream medical community, considerable controversy surrounds "chronic Lyme disease," an ill-defined diagnosis that some clinicians give to patients with alternative diagnoses or medically unexplained symptom complexes. In many instances these patients also are diagnosed with chronic coinfection with *Anaplasma*, *Babesia*, or *Bartonella*. In the context of chronic Lyme disease, these pathogens often are diagnosed in the absence of typical presentations or objective clinical findings, and without laboratory confirmation.

In this systematic review we address several major questions relevant to the diagnosis of coinfections in patients with a diagnosis of chronic Lyme disease. These questions are the following:

1) Is there evidence of persistent human granulocytic anaplasmosis (HGA)?
2) How is relapsing or persisting babesiosis identified and diagnosed?

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Authorship: Both authors had access to all data and took part in writing this manuscript.

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3) Has chronic *Babesia duncani* infection been described?
4) Is there convincing evidence for tick-borne human *Bartonella* infection?
5) Is there convincing evidence for simultaneous Lyme disease and *Bartonella* infection?

METHODS

In order to identify relevant articles, we performed the following Boolean searches of the indexed medical literature using the PubMed search engine.

Search 1

For evidence of chronic human anaplasmosis:

(anaplasma OR anaplasmosis OR ehrlichia OR ehrlichiosis OR phagocytophilum) AND (chronic OR persistent OR recurrent OR relapse)

Search 2

To characterize chronic or relapsing babesiosis:

(babesia OR babesiosis) AND (chronic OR persistent OR recurrent OR relapse)

Search 3

For the role of *Babesia duncani* in human disease:

babesia AND (duncani OR WA1)

Search 4

For tick-borne *Bartonella* infection:

(tick OR Ixodes) AND (bartonella OR bartonellosis)

Search 5

For simultaneous Lyme disease and bartonellosis:

(Lyme OR borrelia OR borreliosis) AND (bartonella OR bartonellosis)

Case reports, case series, and primary scientific studies were selected from among the search results. Review articles, correspondence, and editorials were excluded. We limited our search to studies with human subjects. This was done by manually reviewing the articles and excluding those in which the subjects were nonhuman (rather than adding a search function limit to the PubMed query). Because *Anaplasma phagocytophilum* was formerly categorized as *Ehrlichia*, we included *Ehrlichia* and ehrlichiosis in the search terms for this query.

CLINICAL SIGNIFICANCE

- There is no evidence to support a diagnosis of chronic anaplasmosis in humans.
- Persistent or relapsing babesiosis is accompanied by fever and demonstrable parasitemia.
- There is little evidence to support tick-borne *Bartonella* infection or *Bartonella*-Lyme coinfection.

RESULTS

Search 1: Persistent, Chronic, or Recurrent Human Granulocytic Anaplasmosis

This search yielded 252 articles. The vast majority of scientific articles yielded by these search terms were animal studies. Many addressed microorganisms other than *A. phagocytophilum*. Ultimately, only 2 studies were appropriate for further review based on our inclusion criteria. In the first, 2 febrile asplenic patients were diagnosed with HGA based on blood smear examination.[2] One developed neurologic symptoms including left-sided weakness, left hemi-neglect, and delirium within 12 days of an admission in which HGA had been diagnosed and treated. His blood smear examination was negative at this second visit and he was apparently afebrile, so recurrent HGA was not definitively established; nonetheless, he received doxycycline and promptly improved. The second asplenic patient was treated uneventfully with 10 days of doxycycline and suffered no relapse. A second study reported HGA in 3 recipients of pancreas transplantation.[3] While all of the patients had overall complicated medical courses, none had evidence of recurrent or chronic HGA. This study was reported from Kentucky, a state where HGA is not known to be endemic.

Search 2: Persistent or Relapsing Human Babesiosis

This search yielded 200 articles. Of these, 31 were retrieved for further analysis after screening as described in the Methods section. A large number of these studies documented relapsing or persistent babesiosis or babesiosis whose diagnosis was delayed; complicated disease predominantly affected asplenic or otherwise immunocompromised patients. Fever, laboratory abnormalities such as anemia, and direct evidence of parasitemia such as a positive blood film examination or polymerase chain reaction (PCR) assay were nearly universal among the reported patients.[4-23]

The literature search did not yield evidence of cryptic babesiosis resulting in a less overt syndrome. A study of patients with chronic fatigue syndrome found seroreactivity to *Babesia microti* in 2 controls but not in any of the study subjects with chronic fatigue.[24] A case series of 3 patients attributed panic attacks to infection with multiple tick-borne pathogens including babesiosis.[25] In 2 of the 3 cases, presumption of babesiosis was based solely on antibody titers — an immunoglobulin M (IgM) titer of 1:80 in one case and a "low positive" titer in the other. A third patient in this series reportedly had *B. microti* DNA detected by PCR. Details of the PCR reaction were not provided, there was no report of a blood film examination, and no report of

laboratory testing to evaluate hemolysis; the article reports that the patient's panic attacks were eliminated after 9 months of "increasingly aggressive antimicrobial therapy for tick-borne diseases." None of the antibiotics listed in the article has known efficacy for human babesiosis.

Search 3: *Babesia Duncani* Infection

This search yielded 26 articles. Of these, we identified 13 case reports, case series, or human studies for further review. The remainder was comprised of review material or animal studies. Two instances were reports of a *Babesia divergens*-like pathogen, and infection with *B. duncani* was excluded.

Infection with *B. duncani*, formerly designated WA1, has been described in 8 patients in the medical literature.[26-32] Three of these cases were transfusion-associated. Fever was a predominant symptom in 7 of these cases; this was not the case in that of a premature infant with transfusion-associated disease. In all published cases, infection was directly confirmed by blood smear examination, direct amplification of pathogen DNA, or by inoculation of a laboratory animal. One additional subject from Australia with no history of travel was reported to be positive by PCR for *B. duncani*.[33] His clinical presentation was not described in this publication.

Seropositivity to *B. duncani* appears to be common in asymptomatic individuals. In northern California, 3.5% of all individuals and 16% of higher-risk subjects were seropositive. This was corroborated by a separate study from northern California showing a seroprevalence of 17.8%.[34] Finally, a private reference laboratory reported that 27% of clinical specimens and 2% of specimens from prospective blood donors had titers to *B. duncani* of at least 1:256.[35]

In no published report was *B. duncani* directly detected in afebrile patients who lacked other objective clinical or laboratory signs of disease.

Search 4: Evidence of Tick-borne Human *Bartonella* Infection

A total of 200 articles was identified, the great majority of them reporting the detection of *Bartonella* within ticks. Nine articles were reviewed further for direct evidence of human *Bartonella* infection transmitted by a tick bite, or the vector competence of ticks to transmit *Bartonella* spp. to a host. The most direct evidence of tick-borne human bartonellosis comes from a study of 3 patients from southern France investigating the "scalp eschar and neck lymphadenopathy after tick bite" syndrome.[36] The eschars from 2 of these patients were positive by PCR for *B. henselae*. These patients, however, did not have an identified tick bite, and had other risk factors for *Bartonella* infection (including cat exposure). A third patient had an eschar that was negative by PCR for *B. henselae*. He did, however, provide an ornate sheep tick, *Dermacentor marginatus*, that was retrieved from the site of the eschar; this tick was positive for *B. henselae*. Our search did not yield other articles demonstrating tick transmission of *Bartonella* to humans. Three studies have demonstrated transmission of *Bartonella* spp. by ticks using artificial feeding systems and murine transmission models. One study demonstrated that the brown dog tick, *Rhipicephalus sanguineus*, could become infected with *B. vinsonii* subsp. *berkhoffii* when feeding using a capillary tube system.[37] A second study found that *I. ricinus* ticks could acquire *B. henselae* after feeding on infected blood using a membrane feeding system. Neither of these studies demonstrated transmission of the organism from the tick to a mammalian host. The only study to do so found that *B. birtlesii* could be transmitted to mice by *I. ricinus*.[37-39] This study has not been corroborated by evidence that transmission occurs in nature. No study has yet investigated transmission of *B. henselae* by *I. scapularis*.

Search 5: Evidence of Simultaneous Lyme Disease and *Bartonella* spp. Infection

This search yielded 155 articles, of which 8 were appropriate for further review based on the criteria described in the Methods section. Three of these publications presented patients with putative *Bartonella*/Lyme disease coinfection.[40] One patient had several months of nonspecific symptoms, then sudden vision loss that was attributed to neuroretinitis. Titers were strongly positive to *B. henselae* (>1:1024). The patient had detectable peripheral and cerebrospinal fluid IgM antibodies to *B. burgdorferi*, but had a negative *B. burgdorferi* IgG by established interpretive criteria. The second publication reported 4 symptomatic patients in whom DNA from both *B. henselae* and *B. burgdorferi* were found in the cerebrospinal fluid.[41] Only one of these subjects was seropositive to *B. burgdorferi*. Very little clinical information was given about these patients, including whether there was cerebrospinal fluid evidence of meningitis. Amplicons from PCR reactions were not sequenced. Finally, a third publication reported testing results from 2 patients from Poland with meningitis; no further clinical details were provided in the study.[42] Of these patients, one had *B. henselae* DNA in the cerebrospinal fluid; this individual was seronegative for antibodies to *B. henselae*, but had detectable IgG antibodies to *B. burgdorferi*. A second patient was found to have equivocal levels of antibodies to *B. henselae* and equivocal levels of IgM antibodies to *B. burgdorferi*. Serologic evaluation for Lyme disease in this study did not correspond to current recommendations for 2-tier testing.

A number of other studies have suggested that occupationally exposed individuals are frequently seropositive for antibodies to both *B. burgdorferi* and *Bartonella*. A serosurvey of at-risk individuals in Lublin, Poland (forestry workers and farmers) found that 8.9% of individuals had antibodies to both *Bartonella* spp. and *B. burgdorferi*.[43] A separate study from the Warsaw region found seropositivity to both organisms in 10% of forestry workers.[44] A study of patients with a variety of rheumatic disease manifestations

from a Lyme disease-hyperendemic region found antibodies to *Bartonella* in 62% of subjects and direct detection of the organism in 41.1%; none of these patients, however, had documentation of Lyme disease.[45] Finally, in an Australian study, 2 patients were described as having evidence of simultaneous *Bartonella* infection and Lyme disease. The clinical syndromes from these patients were not described; their seropositivity to *Bartonella* was an isolated IgM titer of 1:40, and these subjects only had IgM seroreactivity to *B. burgdorferi*.[33]

DISCUSSION

There is no debate in the scientific community that *Ixodes* spp. ticks transmit a number of important human pathogens, and sometimes in combination. In addition to *B. burgdorferi*, the causative agent of Lyme disease, *Ixodes* ticks may transmit *B. microti* and other human *Babesia* species, *A. phagocytophilum*, tick-borne encephalitis virus, Powassan virus, and emerging pathogens such as *Borrelia miyamotoi*. These infections may occur in isolation or in various combinations, and it is well established that coinfections have important clinical, diagnostic, and therapeutic implications. Active infection is characterized by objective clinical findings (eg, fever or laboratory abnormalities). Practitioners who frequently offer the diagnosis of chronic Lyme disease often do not rely on more accepted standards of clinical and laboratory testing. In such circumstances, many patients also receive spurious diagnoses of chronic anaplasmosis, babesiosis, and bartonellosis.

We have performed a systematic review of the medical literature in order to evaluate whether published science supports chronic, cryptic infections with these pathogens. Because of basic biological, clinical, and epidemiologic differences among HGA, babesiosis, and bartonellosis, different search terms were required for each pathogen.

A. phagocytophilum, the causative agent of HGA, is a rickettsial organism that produces an acute febrile systemic illness within about 2 weeks of an infectious tick bite. Infection is characterized by fever, constitutional symptoms, and laboratory abnormalities such as leukopenia, thrombocytopenia, and elevated levels of hepatic transaminases. Although HGA is potentially fatal, the infection will be self-limiting in survivors regardless of whether they are treated. As HGA is an infection of circulating leukocytes, both blood film examination and PCR of the blood can establish the presence of infection. Our search did not yield any reports of chronic, relapsing, or refractory HGA in humans. Persistent infection in domestic and wild ruminants, and persistent veterinary infections with related microorganisms (eg, *Anaplasma marginale*) cannot be assumed to predict the plausibility of chronic HGA in humans. To date there is no basis upon which to diagnose a human patient with chronic HGA.

Babesiosis is a malaria-like protozoan infection of erythrocytes that is transmitted by *Ixodes* spp. ticks. It may also be acquired from blood transfusions. Several species of *Babesia* are capable of causing human disease; the most important of these are *B. microti* in the Northeastern and Midwestern US and *B. divergens* in Europe. Lyme-*Babesia* coinfection has been well established and may result in greater disease severity.[10] Clinical babesiosis is nearly always dominated by fever and characteristic laboratory abnormalities, and the infection can be proved by direct visualization of the parasite on blood smear or detection of its DNA by blood PCR.

Relapsing or persistent infection can occur in immunocompromised patients, particularly those with lymphoma who are asplenic and received treatment with rituximab. Persistent babesiosis produces the same clinical and laboratory abnormalities that are seen in acute babesiosis, and patients remain both PCR and blood smear positive. In fact, immunocompromised patients who are at risk of persistent or recurrent babesiosis often have higher parasitemias and generally more severe disease. This is the only group of patients for whom there is evidence that a course of antibabesia drug therapy that exceeds 10 days duration is beneficial.[21] We found no evidence that active babesiosis, as demonstrated by a positive PCR or blood smear, produces purely subjective complaints (eg, fatigue, pain, cognitive symptoms) that are unaccompanied by fever or by laboratory abnormalities. Asymptomatic blood donors have been the index cases for transfusion-associated babesiosis, so it may be the case that patent infection can actually be subclinical or nonspecific. If PCR-negative patients with purely subjective symptoms due to babesiosis exist, there are no published data on whether antibabesia therapy might be beneficial for them. The current standard of care is to treat only those individuals who can be shown by direct molecular or microscopic testing to have active babesiosis.[46] Seroprevalence to *B. microti* clearly exceeds the incidence of clinically evident infections, suggesting that many individuals experience subclinical and asymptomatic infections. Thus, reliance on serology in the absence of direct demonstration of the organism could lead to erroneously attributing coincident symptoms to active infection.

This is particularly true for *B. duncani*, a pathogen responsible for a small number of human cases in the Pacific Northwest. Like other human babesias, *B. duncani* produces fever and hemolysis. Among the limited case reports there was no evidence of cryptic infection resulting only in subjective complaints. The high rates of background seropositivity to *B. duncani*, including in supposedly nonendemic areas according to one report, raise the question of whether there are cross-reactive antibodies in the population at large. This underscores the importance of directly demonstrating intraerythrocytic infection when pursuing a diagnosis of active babesiosis.

Unlike HGA and babesiosis, which in nature are exclusively transmitted to humans by *Ixodes* spp. ticks, we have found no convincing evidence that this is a natural or even plausible mode of transmission for *Bartonella* spp. Our search yielded no case in which tick-borne bartonellosis was

unequivocally established. Not only is tick-borne human bartonellosis unfounded to date, but there is very little literature to support Lyme disease−*Bartonella* coinfection at all, regardless of the means of acquisition. Moreover, appropriate seroepidemiologic studies have not even been attempted in Lyme disease patients in the US to evaluate the seroprevalence of *B. henselae* in such individuals. While several small case series and reports in the literature purport to describe simultaneous Lyme disease and *Bartonella* infection, in no case did the laboratory corroboration of Lyme disease correspond to established diagnostic standards.

The putative association between ticks, Lyme disease, and *B. henselae* infection is ultimately derived from 2 problematic sources of data. The first is a limited number of reports of mostly European subjects in whom clinical infection with *B. henselae* and *B. quintana* has been temporally associated with a tick bite.[47-49] The second source of data is the observation that many tick specimens contain *Bartonella* DNA when subjected to PCR analysis.[50-54] This has been demonstrated primarily in the Eurasian ticks *I. ricinus* and *I. persulcatus*, and to a lesser degree, in the North American tick *I. scapularis*. Nonetheless, it should come as no surprise that ticks would contain *Bartonella* DNA − ticks feed on a variety of mammalian hosts that may be reservoirs for *Bartonella* spp. The presence of *Bartonella* DNA in the tick does not prove that the tick is a competent vector for transmission to a second mammalian host. Vector competence of *I. scapularis* ticks for *B. henselae* has never been demonstrated in an animal system.

CONCLUSION

The *Ixodes* spp. ticks that transmit *B. burgdorferi* are capable vectors of several human pathogens. In all cases, however, these infections produce defined clinical syndromes that are corroborated by objective clinical and laboratory findings. This is true for well-established *Babesia*-Lyme and *Anaplasma*-Lyme coinfections. Treatment and diagnosis of chronic coinfections, however, is clearly not justifiable in the absence of convincing objective evidence that these infections are present and active.

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Functional significance of CD57 expression on human NK cells and relevance to disease

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Historically, human NK cells have been identified as $CD3^{-}CD56^{+}CD16^{\pm}$ lymphocytes. More recently it has been established that CD57 expression defines functionally discrete sub-populations of NK cells. On T cells, CD57 expression has been regarded as a marker of terminal differentiation and (perhaps wrongly) of anergy and senescence. Similarly, CD57 expression seems to identify the final stages of peripheral NK cell maturation; its expression increases with age and is associated with chronic infections, particularly human cytomegalovirus infection. However, $CD57^{+}$ NK cells are highly cytotoxic and their presence seems to be beneficial in a number of non-communicable diseases. The purpose of this article is to review our current understanding of CD57 expression as a marker of NK cell function and disease prognosis, as well as to outline areas for further research.

Keywords: CD57, NK cells, HCMV infection, ageing, chronic infection, cancer, autoimmune diseases, T cells

CD57 IS A MARKER OF NK CELL DIFFERENTIATION

CD57 was first identified on cells with natural killer activity using the mouse monoclonal antibodies Human Natural Killer-1 (HNK-1) (1) and Leu-7 (2) and was subsequently assigned the cluster of differentiation (CD) designation, CD57, at the fourth International Workshop of Human Leukocyte Antigens in 1989. HNK-1/Leu-7/CD57 was initially believed to be uniquely expressed on NK cells – and was used to define this population (1, 3) – although it was soon apparent that CD57 was expressed only on a subset of functionally distinct NK cells (4). CD57 was subsequently identified on $CD8^{+}$ T cells (5–7) as well as cells of neural crest origin (1, 8–13). Indeed, it was the neuroscience community that ultimately defined CD57 as a terminally sulfated carbohydrate epitope (glucuronic acid 3-sulfate) (14–16). In neural cells, the CD57 epitope is predominantly restricted to adhesion molecules (17) but little attention has been paid to the precise identity of the molecules expressing the CD57 epitope on NK cells and T cells, precluding a full understanding of the relationship between CD57 expression and lymphocyte function. Although one study identified the CD57 epitope on the IL-6 receptor gp130 of resting lymphocytes (18), the cells expressing CD57/gp130 were not identified and no comprehensive analysis of CD57-expressing molecules on T cells or NK cells has been reported.

While first characterized as an NK cell marker, CD57 has been most widely explored as a marker of replicative senescence on T cells (19). Under conditions of persistent immune stimulation, memory T cells convert from $CD28^{+}CD57^{-}$ to $CD28^{-}CD57^{+}$ (20); $CD57^{+}$ cells have short telomeres, low telomerase activity, low expression of cell-cycle associated genes and limited proliferative capacity (20, 21). However, $CD57^{+}CD28^{-}CD8^{+}$ T cells can proliferate given an appropriate cytokine milieu (22), their sensitivity to apoptosis is disputed (23, 24), they are highly cytotoxic (25, 26) and express natural killer receptors (27). $CD57^{+}CD8^{+}$ T cells should thus be regarded as terminally differentiated, oligoclonal populations of cytotoxic cells generated in response to chronic antigen stimulation.

In light of the T cell data it was suggested that CD57 may also be a marker of NK cells with poor proliferative capacity and, perhaps, a degree of immunosenescence (21, 23, 28). Indeed, acquisition of CD57 on NK cells – following stimulation with IL-2 or coculture with target cells – correlates with maturation of the $CD56^{dim}$ NK cell subset, with lower expression of NKp46, NKp30, NKG2D, and NKG2A, and higher expression of CD16, LIR-1, and killer cell immunoglobulin-like receptors (KIRs) (29). Similarly, in hematopoietic stem cell transplant recipients exposed to human cytomegalovirus (HCMV) infection, differentiation of $CD56^{dim}$ NK cells involves acquisition of CD57, loss of NKG2A, gain of KIRs, and changing expression of homing molecules (30). These studies, together with experiments in $Rag2^{-/-}\gamma cR^{-/-}$ mice reconstituted with human hematopoietic stem cells and treated with IL-15 (30), and the observation that fetal and newborn NK cells lack CD57 (31), indicate that $CD57^{+}$ NK cells differentiate from $CD56^{dim}CD57^{-}$ NK cells in an irreversible process with highly stable expression of CD57 likely being the final step in maturation (30, 32). This differentiation is accompanied by functional changes (29, 30): compared with $CD57^{-}$ cells, $CD57^{+}$ NK cells proliferate less well in response to IL-2 and IL-15 and produce less IFN-γ in response to IL-12 and IL-18, consistent with their lower levels of IL-12Rβ mRNA (29) and reduced surface expression of IL-2Rβ and IL-18Rα (30). On the other hand, $CD57^{+}$ NK cells retain their cytolytic potential (30) and a proportion of $CD57^{+}$ NK cells are able to produce IFN-γ after crosslinking of CD16 [Ref. (29); White et al. submitted] indicating that $CD57^{+}$ NK cells are intrinsically able to produce IFN-γ but that they may have different activation requirements.

In summary, therefore, progression from $CD56^{bright}$ to $CD56^{dim}CD57^{-}$ to $CD56^{dim}CD57^{+}$ reflects a maturation pathway for NK cells (33, 34) and rather than being a marker of anergy or

immunosenescence, acquisition of CD57 represents a shift toward a higher cytotoxic capacity, greater responsiveness to signaling via CD16 and natural cytotoxicity receptors (NCRs) and decreased responsiveness to cytokines (29, 35). The extent to which CD57 expression *per se* drives these changes in function, as opposed to being a marker for cells with altered expression of other attributes of a mature NK cell, is not entirely clear and may represent a fertile area for further research. In addition, a much better characterization is required of the cell surface molecules that express the CD57 epitope, the mechanisms by which CD57 is induced on them, and its functional consequences.

CD57 EXPRESSION AND CANCER

Both $CD8^+$ T cells and NK cells are able to kill tumor cells through mechanisms including perforin/granzyme-mediated cytolysis and TRAIL- or FAS-mediated apoptosis (36). Accumulation of $CD57^+CD8^+$ T cells is seen frequently in individuals with various forms of cancer (37) and has been associated with reduced survival in those with renal cell carcinoma (38), melanoma (39), gastric carcinoma (40), multiple myeloma (41), lymphomas, acute and chronic myeloid, and lymphocytic leukemias (42), among many other examples. CD57 expression on $CD4^+$ T cells has also been associated with Hodgkin's lymphoma (43) and chronic lymphocytic leukemia (44). This association between malignancy and expanded populations of $CD57^+$ T cells is likely explained by persistent stimulation of these cells by tumor-associated antigens in the absence of effective tumor clearance (45).

NK cells were initially identified by their ability to kill malignant cells (46–48) and a large body of clinical and experimental evidence now supports their crucial role in cancer immunosurveillance (49). Reduced MHC Class I expression (50) and *de novo* expression of stress related molecules (such as B7-H6, MICA, MICB, RAE-1, MULT1, and members of the ULBP family) in malignant cells alter the balance of inhibitory (via KIRs and NKG2-CD94 heterodimers) and activating (via NCRs and NKG2D homodimers) signals for NK cells (51), leading to their activation. High frequencies of peripheral or tumor-associated $CD57^+$ NK cells are reported in cancer patients and – in sharp contrast to what has been seen for $CD8^+$ T cells – have frequently been linked to less severe disease and better outcomes (**Table 1**). This would be consistent with enhanced tumor surveillance/cytotoxicity of the mature, $CD57^+$ NK cell subset (29); whether these associations are confounded by HCMV infection status (see below) is currently unclear. In the case of advanced gastrointestinal stromal tumors treated with the chemotherapeutic agent imatinib mesylate, NK cell secretion of IFN-γ after IL-12/IL-2 stimulation was correlated with improved long-term survival (52). Since $CD57^-$ NK cells are the major subset producing IFN-γ in response to cytokines, this suggests that a heterogeneous NK cell population comprising both $CD57^-$ and $CD57^+$ subsets may be optimal for combating neoplasia. Clearly further studies, ideally longitudinal in nature and accompanied by data on potentially confounding factors, are needed to determine the roles of different NK cell subsets in combating different types of malignancies.

CD57 EXPRESSION AND AUTOIMMUNITY

Autoimmune diseases tend to be highly antigen-specific and mediated by autoantibodies or autoreactive T cells. In general, expanded populations of autoreactive $CD57^+$ T cells are associated with more severe disease – Wegener's granulomatosis (65), pars planitis (25), multiple sclerosis (MS) (66), type I diabetes mellitus (67), Graves' disease (68), and rheumatoid arthritis (RA) (69), amongst others. This likely reflects killing of vital host cells by these highly cytotoxic lymphocytes (68), although the loss of T cells with immunosuppressive potential may also play a role (67).

Perhaps surprisingly, autoimmune disease is consistently associated with reduced frequencies or absolute numbers of circulating $CD57^+$ NK cells and/or impaired NK cell cytotoxicity (**Table 2**) (70–78), suggesting that cytotoxic $CD57^+$ NK cells may play a regulatory role, preventing or suppressing autoimmune disease. In MS, peripheral NK cells lose expression of FAS during relapse and regain it during remission (70) and FAS^+ NK cells can inhibit myelin basic protein-specific T cell IFN-γ responses (79), suggesting that NK cells may regulate autoreactive T cells. On the other hand, chronic NK cell lymphocytosis (which is associated with peripheral neuropathy, arthritis, and vasculitis) is characterized by increased absolute numbers of circulating immature NK cells with low cytotoxicity (80, 81). Similarly, NK cells have been found in the inflammatory infiltrates of psoriatic skin lesions (82), in synovial fluid of joints affected by RA (83), and in pancreatic islets of type I diabetes patients (84). NK cells in the synovial fluid of patients with RA, and those infiltrating psoriatic skin lesions, are immature $CD56^{bright}$ or $CD57^-$ and able to secrete IFN-γ and TNF (85, 86), suggesting that they may contribute to the inflammation rather than suppress it (84).

Taken together, these data are consistent with the hypothesis that immature $CD57^-$ NK cells may contribute to autoimmune inflammation and tissue damage whereas more highly differentiated, cytotoxic, $CD57^+$ NK cells may fulfill an immunoregulatory role, possibly deleting chronically activated T cells, as in viral hepatitis (103).

CD57 EXPRESSION DURING INFECTION

Chronic viral infections such as HCMV (104), human immunodeficiency virus (HIV) (105), hepatitis C virus (106), and Epstein–Barr virus (EBV) (107) infections offer some of the clearest examples of expansion of $CD57^+CD8^+$ T cells, presumably as a result of persistent antigenic stimulation, and increased proportions of $CD57^+CD8^+$ T cells have also been reported in those infected with human parvovirus (108), measles (109), pulmonary tuberculosis (92), and toxoplasmosis (93). The majority of these $CD57^+CD8^+$ T cells, at least in HCMV infection, appear to be antigen-specific and their presence is associated with a low incidence of reactivation (94, 95). Similar skewing of NK cells toward the $CD57^+$ phenotype is now reported in a variety of viral infections (**Table 2**).

Increased frequencies of $CD57^+CD16^+$ NK cells were first reported in HCMV-infected individuals by Gratama et al. (110) and have been repeatedly confirmed (99, 111, 112). Studies of hematopoietic stem cell transplantation (HSCT) have been particularly informative, allowing detailed comparison of stem cell differentiation into NK cells in HCMV-infected and uninfected transplant recipients (111, 112) with rapid and persistent expansion of $CD57^+$ NK cells that are also $NKG2C^+$, KIR^+, $CD158b^+$, and potent producers of IFN-γ after stimulation with MHC Class I-deficient target cells, only in the HCMV-infected group (111). We now know that HCMV drives expansion of $NKG2C^+$ NK cells and

Table 1 | Associations between cancer prognosis and CD57 expression by NK cells.

Cancer type	Observations	Reference
Acute lymphoblastic leukemia	Increased NK cell activity and increased numbers of $CD57^+$ and $CD16^+$ NK cells in bone marrow associated with complete remission	Sorskaar et al. (57)
Hodgkin's disease	Absence/low number of $CD57^+$ NK cells in tumor tissue (by immunohistochemistry) associated with relapse	Ortaç et al. (58)
Non-Hodgkin's lymphoma	Higher numbers of intratumoral $CD57^+$ NK cells are associated with relapse free survival in pediatric cases	Ortaç et al. (58)
Metastatic tumors in the brain	$CD57^+$ NK cells infiltrate brain metastases of various origins (lung, breast, and renal carcinomas; melanoma) but no correlation between numbers of infiltrating $CD57^+$ NK cells and apoptosis of malignant cells	Vaquero et al. (59)
Colorectal cancer	Increased $CD57^+$ NK cells in germinal centers of draining lymph nodes, but rarely in primary or metastatic lesions; $CD57^+$ NK cells may prevent establishment of tumor in lymph nodes?	Adachi et al. (60)
Bladder carcinoma	Lower frequency of $CD56^+$ and $CD57^+$ PBMC in patients with invasive and non-invasive tumors is correlated with reduced cytotoxicity against T24 bladder cancer cell line	Hermann et al. (61)
Breast carcinoma	Survival is positively correlated with the number of tumor infiltrating $CD57^+$ NK cells and with expression of CX3CL1 (a known NK cell chemoattractant) by the tumor cells	Park et al. (62)
Gastric carcinoma	$CD57^+$ NK cell infiltration associated with a lower clinical grade tumor, reduced venous invasion, fewer lymph node metastases, less lymphocytic invasion, and increased 5 year survival outcome	Ishigami et al. (63)
Oral squamous cell carcinoma	Low density of tumor infiltrating $CD57^+$ NK cells and high numbers of TNF^+ cells associated with higher clinical staging	Turkseven and Oygur (64)
Esophageal squamous cell carcinoma	Tumor infiltrating $CD57^+$ NK cells positively associated with increased survival over 80 months	Lv et al. (87)
Squamous cell lung carcinoma	Tumor infiltrating $CD57^+$ NK cells positively correlated with increased survival 2 years after surgery	Villegas et al. (88)
Pulmonary adenocarcinoma	Higher absolute numbers of tumor infiltrating $CD57^+$ NK cells correlated with tumor regression	Takanami et al. (89)
Various	Low numbers of $CD57^+$ NK cells in peripheral blood are associated with carcinomas of colon, lung, breast, and neck; no association was with melanoma or sarcoma	Balch et al. (90)

that these cells preferentially acquire CD57 (97–99, 111, 112). In HCMV-uninfected donors, there are roughly equal proportions of $CD57^+NKG2C^+$ and $CD57^-NKG2C^+$ NK cells whereas the ratio of $CD57^+NKG2C^+$ to $CD57^-NKG2C^+$ NK cells ranges from <1 to >60 in HCMV-infected donors (99); whether this variation reflects varying duration of HCMV infection is not known. HCMV reactivation after HSCT is associated with a threefold increase in the ratio of $CD57^+NKG2C^+$ to $CD57^-NKG2C^+$ NK cells within one year (111). Yet, in the absence of HCMV infection, $NKG2C^+$ NK cells are no more likely to acquire CD57 than are $NKG2C^-$ NK cells (112), suggesting that either binding of NKG2C to specific HCMV ligands or chronic viral infection *per se* drives NK cell differentiation. Importantly, $CD57^+CD16^+$ NK cells can kill HCMV-infected target cells (96) and this may be dependent upon, or enhanced by, α-HCMV antibodies (113).

While HCMV remains the clearest example of infection driving NK cell differentiation, other viral infections may cause a similar effect. For example, there is a three to fourfold expansion of the NK cell pool during acute hantavirus infection; NK cell numbers peak approximately 10 days after the onset of symptoms and remain above baseline for at least 60 days (114). This expansion is restricted to the $NKG2C^+$ NK cell subset and the majority of these cells are $CD57^+$, KIR^+ and highly responsive to MHC Class I-deficient target cells. Hantavirus-infected endothelial cells express high levels of the NKG2C ligand HLA-E and expansion of the $NKG2C^+$ NK cell subset is seen only in HCMV seropositive hantavirus patients, suggesting that hantavirus-induced HLA-E expression and/or inflammatory cytokines released during infection may drive the expansion and subsequent maturation of $NKG2C^+$ NK cells that have been induced or "primed" by HCMV infection (114). Similarly, transient expansion of the $CD57^+$ $NKG2C^+$ NK cell population during acute chikungunya virus infection is also associated with HCMV seropositivity (115).

Expansion of the $NKG2C^+CD57^+$ NK cell subset has also been reported in $HCMV^+$ individuals with chronic hepatitis B and hepatitis C infections, although the proportions of these cells did not differ markedly from previous reports in HCMV-infected but hepatitis virus-uninfected donors, leading the investigators to conclude that HCMV, rather than viral hepatitis, is the underlying driver of NK cell differentiation (97). In line with this, no

Table 2 | Associations between autoimmune diseases or infections and CD57 expression by NK cells.

	Observations	Reference
AUTOIMMUNE DISEASE		
Alopecia areata	$CD57^+$ NK cells are significantly reduced in peripheral blood of patients with multiple foci of alopecia	Imai et al. (91)
Atopic dermatitis	Reduced frequencies of $CD57^+$ NK cells in peripheral blood of patients compared to healthy controls, with greatest reduction in the most severe cases	Wehrmann et al. (126) and Matsumura (127)
Sjögren's syndrome	Decreased numbers of $CD57^+$ NK cells observed in peripheral blood of patients compared to controls	Struyf et al. (128)
IgA nephropathy	Decreased proportion of $CD57^+$ $CD16^+$ lymphocytes in the peripheral blood of patients compared to healthy controls	Antonaci et al.(129)
Psoriasis	NK cells infiltrating skin lesions – but also unaffected skin – are predominantly CD57low	Batista et al. (85)
INFECTION		
HCMV	Increased proportions of $CD57^+$ NK cells in infected individuals; CD57 expression limited to the $NKG2C^+$ subset	Gratama et al. (110), Lopez-Vergès et al. (99) and Foley et al. (111, 112)
HIV	In chronic infections, there is a loss of CD57-/dim NK cells, but the absolute number of $CD57^+$ NK cells remains constant	Hong et al. (100)
Chikungunya virus	Increased proportions of $CD57^+$ NK cells after infection in $HCMV^+$ patients	Petitdemange et al. (115)
Hantavirus	$NKG2C^+$ NK cell subset expanded during infection in $HCMV^+$ patients and the majority of these cells are $CD57^+$	Björkström et al. (114)
Hepatitis B and Hepatitis C	$NKG2C^+$ NK cell population is expanded in chronic infections, and these are predominantly $CD57^+$, but co-infection with HCMV appears to be the driver of this effect	Béziat et al. (97)
Lyme disease	Conflicting evidence on whether chronic disease leads to a reduced proportion of $CD57^+$ NK cells in peripheral blood	Stricker et al. (117), Stricker and Winger (118), and Marques et al. (119)

association was found between expansion of the $NKG2C^+CD57^+$ NK cell subset and clinical indicators of hepatitis such as viral load or liver enzyme concentrations (97).

In HIV-infected individuals, the absolute number of $CD57^+$ NK cells is stable and comparable to HIV-negative individuals but the ratio of $CD57^+$ to $CD57^-$ NK cells is higher than in uninfected individuals due to a gradual loss of $CD57^-$ cells (which are highly dependent on monocyte and T cell-derived cytokines for their survival) (100). Unfortunately, the HCMV status of these subjects was not reported and may confound the comparison between the HIV^+ and HIV^- individuals. Indeed, in another study, the positive association between frequency of $NKG2C^+$ NK cells and HIV-1 infection disappears when adjusted for HCMV status (101). Nonetheless, it is also the case that the frequency of $NKG2C^+(CD57^+)$ NK cells is higher in HCMV seropositive donors with HIV-1 infection than in HCMV seropositive donors without HIV-1 infection (102), suggesting either that – as for hantavirus or chikungunya virus – HIV-1 infection drives expansion of the HCMV-induced $NKG2C^+$ population or that HIV-1 infected individuals experience more frequent reactivation of HCMV which then expands the $NKG2C^+$ population. Significantly, $CD57^+$ NK cells of HIV^+ individuals retain a highly differentiated phenotype ($CD16^+KIR^+perforin^+$) but have defects in degranulation (100) suggesting that they may have reduced cytotoxic potential. Finally, although no association was seen between accumulation of $CD57^+$ NK cells and recurrence of genital herpes lesions due to herpes simplex virus 2 (HSV-2) infection (116), interpretation of this study is hindered by the lack of an HSV-2-uninfected control group.

There have been very few studies of NK cell subsets in the context of bacterial or parasitic infections. Patients with chronic Lyme Disease (*Borrelia burgdorferi*) have lower proportions of peripheral blood $CD57^+$ NK cells compared to those with acute disease and uninfected controls and this phenotype was maintained for over 10 years in one person with persistent infection (117, 118). In contrast, no significant differences in numbers of peripheral blood $CD3^-CD57^+$ cells were noted between patients with post-Lyme disease syndrome, individuals recovered from Lyme disease and healthy controls (119). The suggestion (118) that high frequencies of $CD57^+$ NK cells may be a biomarker of Lyme disease progression thus seems premature, especially given the potential impact on NK cell phenotype of HCMV and other infections.

In summary, viral infections are important drivers of NK cell differentiation with HCMV playing a primary role in selecting for $NKG2C^+CD57^+$ cells and other viruses driving their expansion and differentiation.

CD57 EXPRESSION AND AGING

Given the enormous impact of infection on NK cell maturation and differentiation, it is not surprising that NK cell populations change with age, which is a proxy for cumulative exposure to infection and other physiological insults. At birth virtually no T cells express CD57 (120) but the proportion rises with age, reaching 20–30% in young adults (20); by 80 years of age 50–60% of $CD8^+$ T cells are $CD28^-$ (and thus likely $CD57^+$) (20, 121). Similarly, with increasing age, increasing numbers of circulating NK cells are achieved by an expansion of the $CD56^{dim}$ and $CD57^+$ subsets and an absolute, as well as a proportional, decline in $CD56^{bright}$ cells (35, 53–55, 122–125). At birth, all $CD56^{dim}$ NK cells are $CD57^-$; among European adults (18–60 years of age) 25–60% of $CD56^{dim}$ NK cells are $CD57^+$ and this continues to increase slightly, but significantly, after the age of 80 years (124). Interestingly, $CD56^{dim}CD57^+$ NK cells accumulate very rapidly in an African (Gambian) population reaching adult levels (20–70%) by the age of 5 years (Goodier et al. unpublished); this may reflect very high HCMV seroprevalence rates in this age group in this community.

The increased proportion of $CD56^{dim}CD57^+$ NK cells in the elderly likely explains the maintenance of NK cell cytotoxic responses despite reduced responsiveness to cytokine stimulation [reviewed in Ref. (56)], however, the significance of these changes in terms of overall immune competence is poorly understood. The gradual loss of the $CD56^{bright}$ NK cell population, and the consequent decline in NK-derived cytokines that activate dendritic cells and monocytes, has been assumed to contribute to age-associated declines in immune competence but the potential counterbalancing effect of an increased proportion of highly cytotoxic $CD57^+$ NK cells has received little attention (123). Comprehensive studies are now needed to assess the cytokine-producing and cytotoxic function of individual NK cell subsets in response to cytokine stimulation as well as activation via CD16 and NCRs and the extent to which this changes with age and HCMV status.

CONCLUSION AND FUTURE DIRECTIONS

CD57 is a very useful marker of NK cell maturation, identifying cells with potent cytotoxic potential but decreased sensitivity to cytokines and reduced replicative potential. $CD57^+$ NK cells appear to be a stable sub-population, increasing with age and exposure to pathogens (especially, but not exclusively, HCMV) and their presence is consistently associated with better outcomes in cancer and autoimmune disease. However, the majority of clinical studies have been cross-sectional, with limited follow up and data on crucial confounding factors such as HCMV infection are typically lacking. Recent studies of HSCT (111, 112) demonstrate the power of prospective and longer term studies in beginning to assign causality in terms of NK cell phenotype, function, and disease. Nevertheless, precise understanding of the role of CD57 expression on NK cells requires a detailed dissection of the underlying biology of CD57, about which very little is known. Given that there is no evidence that CD57 is expressed on murine NK cells, this is not a simple task. Possible approaches in human NK cells might include conducting a comprehensive analysis of NK cell molecules expressing CD57, blocking CD57 in *in vitro* functional NK cell assays, or manipulating expression or enzymatic activity of B3GAT1 (the key enzyme in the biosynthesis of CD57) using RNA interference or specific inhibitors.

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hypothesis that unobserved patient characteristics are responsible for lower cardiac risk among patients taking atenolol.

It instead tests whether there is an association between atenolol use and risk factors for retained instruments such as obesity, unplanned or altered operations,[2] and staff adherence to operative checklists. However, these biases are not likely to coexist with other unobserved patient characteristics that confound the association between atenolol use and perioperative cardiac risk (eg, socioeconomic or clinical characteristics not accounted for in the analysis).

Therefore, a test of whether atenolol use is associated with higher rates of retained foreign objects after surgery would not be a useful falsification test.

We have centered our discussion on an observational study of perioperative β blockade, but note that in this particular case, we do not need to rely on observational evidence. A well-done randomized trial has tested whether long-acting perioperative β blockade is better than placebo and yielded negative results.[3]

In short, in much the same way observational studies are useful only when used appropriately (eg, when prospective designs are not feasible), falsification testing is valuable only if and when it is applied thoughtfully.

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RESEARCH LETTER

Serologic Markers of Lyme Disease in Children With Autism

To the Editor: A proposed link between Lyme disease and autism has garnered considerable attention.[1,2] Among individuals with autism spectrum disorders, rates of seropositivity for Lyme disease of greater than 20% have been reported.[1] However, controlled studies to assess serological evidence of infection with *Borrelia burgdorferi* (the causative agent of Lyme disease) in patients with autism are lacking.

Serological evidence of infection with *B burgdorferi* is essential for diagnosing Lyme disease, except in cases of typical erythema migrans skin lesions. To evaluate the suggestion that autism is commonly linked to Lyme disease, we performed Lyme disease serological testing on serum samples from children with autism and those without autism.

Methods. Serum samples from 120 children aged 2 through 18 years with autism and those without autism were acquired from the Autism Genetic Resource Exchange (AGRE) (37 with autism and 27 unaffected siblings) and the Weill Cornell Autism Research Program (WCARP) (33 with autism, 8 unaffected siblings, and 15 unrelated healthy controls). All WCARP and some unselected AGRE sites collected serum samples; all available serum samples were included.

Patients from the AGRE program met diagnostic criteria for autism based on both the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview, Revised, whereas WCARP patients met criteria for autism based only on the ADOS. Participants in the AGRE program have been recruited primarily from the northeastern and western United States; serum samples for this study were collected from August 31, 1999, through April 25, 2001.

The WCARP serum samples were from participants who resided primarily in Connecticut, New Jersey, and New York, and were collected from May 19, 2010, through March 7, 2012. Screening questionnaires were used to evaluate the general health of unrelated controls.

Written informed consent was obtained for all study participants from a parent or guardian. Serum samples from 2 patients with culture-confirmed early Lyme disease were used as positive controls. Specimens were kept at −80°C to maintain stability. This study was approved by the institutional review board of Columbia University Medical Center.

Testing for antibodies to *B burgdorferi* was performed according to the 2-tier algorithm recommended by the US Centers for Disease Control and Prevention.[3] Initial screening for anti–*B burgdorferi* immunoglobulin G and M antibodies was performed with separate enzyme-linked immunosorbent assays (ELISAs), according to the manufacturer's protocols (Euroimmun). Specimens classified as borderline or positive were further tested by Western blotting for IgG or IgM antibodies to electrophoresis-separated *B burgdorferi* strain B31 proteins (Euroimmun).[4]

Assuming 1% or lower seroprevalence in controls, and at least 20% seroprevalence in cases as suggested, the sample size in this study would provide greater than 90% power with an α level of .05. Differences between groups were analyzed using the 2-tailed Fisher exact test; *P* values of less than .05 were considered to be statistically significant. Binomial distribution confidence intervals were determined by the Clopper-Pearson exact method.

Results. Seventy children with autism (58 male; mean [SD] age, 7.2 [3.6] years) and 50 unaffected controls (32 male; mean age, 9.0 [4.0] years) were included. Of the patients with autism, 1 was positive by ELISA for anti–*B burgdorferi* IgG, whereas 4 were borderline by ELISA for IgM. Of the 50 children in the unaffected control group, 4 were positive and 1 was borderline for IgG by ELISA, whereas 1 was positive by ELISA for IgM.

All serum samples that were positive or borderline by ELISA were further analyzed using Western blot and were found to be negative for anti–*B burgdorferi* antibody reactivity (**Table 1** and **Table 2**). The 95% confidence interval for seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.

Discussion. None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. A potential limitation of this study is the lack of information about lifestyle for patients and controls, including time spent outdoors.

The data do not address whether Lyme disease may cause autism-like behavioral deficits in some cases. However, the study's sample size is large enough to effectively rule out the suggested high rates of Lyme disease or associated seroprevalence among affected children.

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Table 1. Serum Immunoglobulin G Antibody Reactivity to *Borrelia burgdorferi* Protein Bands as Determined by Western Blotting in Patients and Controls Who Were Positive or Borderline for IgG by Enzyme-Linked Immunosorbent Assay[a]

Serum Sample No.	Group	Western Blot Band[b]											
		p18	p25	p28	p30	p31	p34	p39	p41	p45	p58	p66	p93
1	Autism[c]								+			+	
2	Unaffected control[c]				+				+				
3	Unaffected control[c]								+				
4	Unaffected control[c]								+		+		
5	Unaffected control[c]	+							+			+	+
6	Unaffected control[c]								+				
12	Lyme disease control[d]	+	+					+	+	+		+	
13	Lyme disease control[c]		+						+	+			

[a] None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. The 95% confidence interval for IgG seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.
[b] According to Centers for Disease Control and Prevention testing criteria, an IgG immunoblot was considered positive if 5 or more of the 10 following protein bands reacted positively: p18, p25 (OspC), p28, p30, p39 (BmpA), p41(FlaB), p45, p58, p66, and p93.[3]
[c] Individual did not meet IgG seropositivity criteria for Lyme disease.
[d] Individual met IgG seropositivity criteria for Lyme disease.

Table 2. Serum Immunoglobulin M Antibody Reactivity to *Borrelia burgdorferi* Protein Bands as Determined by Western Blotting in Patients and Controls Who Were Positive or Borderline for IgM by Enzyme-Linked Immunosorbent Assay[a]

Serum Sample No.	Group	Western Blot Band[b]		
		p25	p39	p41
7	Autism[c]			+
8	Autism[c]			+
9	Autism[c]			+
10	Autism[c]			
11	Unaffected control[c]			
12	Lyme disease control[d]	+		+
13	Lyme disease control[d]	+	+	+

[a] None of the children with autism or unaffected controls had serological evidence of Lyme disease by 2-tier testing. The 95% confidence interval for IgM seroprevalence in children with autism and in unaffected controls was 0% to 5.1%.
[b] According to Centers for Disease Control and Prevention testing criteria, an IgM immunoblot was considered positive if 2 of the 3 following protein bands reacted positively: p25 (OspC), p39 (Bmp A), and p41 (FlaB).[3]
[c] Individual did not meet IgM seropositivity criteria for Lyme disease.
[d] Individual met IgM seropositivity criteria for Lyme disease.

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Study concept and design: Alaedini.
Acquisition of data: Ajamian.
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CORRECTIONS

Incomplete Conflict of Interest Disclosures: In the Preliminary Communication entitled "Effects of Fructose vs Glucose on Regional Cerebral Blood Flow in Brain Regions Involved With Appetite and Reward Pathways" published in the January 2, 2013, issue of *JAMA* (2013;309[1]:63-70), information reported by the authors for the Conflict of Interest Disclosures section was inadvertently omitted. The text in that section should have read as follows: "All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Sinha reported receiving a grant from and being a consultant to the National Institutes of Health; and being a scientific advisory board member for Embera Neurotherapeutics. Dr Sherwin reported receiving grants from the National Institute of Diabetes and Digestive and Kidney Diseases and the Juvenile Diabetes Research Foundation; serving on scientific advisory boards for Amylin Corp, Merck, Pfizer, Janssen, and Insulet; serving on data and safety monitoring boards for Novartis and MannKind; being a consultant to Bristol-Myers Squibb, Eli Lilly, and McKinsey & Company; owning stock in Insulet; and receiving payment for lectures from Merck. Dr Page reported no disclosures." This article has been corrected online.

Clarification of Statement: In the Editorial entitled "Promoting Quality Surgical Care: The Next Steps" published in the February 27, 2013, issue of *JAMA* (2013; 309[8]:827-828), a statement requires clarification. In the fourth full paragraph, the last sentence of this paragraph should read "The CMS already requires that Medicare beneficiaries receive care at institutions accredited by approved accreditation organizations such as the Joint Commission." This article has been corrected online.

Lyme Borreliosis is not Sexually Transmitted

From past experience as a research scientist and editor for various scientific journals, I appreciate the fact that abstracts presented at scientific meetings sometimes consist of rather exciting - but still very preliminary - findings that are not always reproducible. This is why abstracts are not given the same consideration as peer-reviewed publications and cited in the bibliographies of scientific papers. There are times when one must be extremely skeptical of their credibility, especially when they contradict the results of published, peer-reviewed research. I refer specifically to the recent abstract by M.J. Middelveen et al. (1), suggesting that Lyme disease may be a sexually transmitted infection, a suggestion based solely on the unconfirmed detection of Borrelia in the semen and vaginal secretions of only three people .

The concept of sexual transmission of borreliosis was refuted years ago by the well-designed and controlled studies of Moody and Barthold (2), as well as Woodrum and Oliver (3), internationally known experts on Lyme disease. These investigators used well-characterized animal models of borreliosis in which infection is much more disseminated and profound than it is in humans. It should be noted that, in the United States, Lyme borreliosis has historically been defined as a tick borne infection caused by *Borrelia burgdorferi sensu lato* (4).

To determine if borreliosis can be transmitted by direct contact, Moody and Barthold (2) housed three-day-old - or three-week-old - Lewis rats, deliberately infected with *B. burgdorferi*, with normal, uninfected rats for 30 days. As expected, all deliberately infected rats continued to be actively infected, 30 days later; however, none of the uninfected rats acquired infection after 30 days of intimate direct contact with their infected housemates. In other experiments, Moody and Barthold (2) were unable to demonstrate venereal transmission of borreliosis from seven infected females - or six infected males - to uninfected rats of the opposite sex.

In the work of Woodrum and Oliver (3), six female Syrian hamsters infected with *B. burgdorferi* were mated with six uninfected males; conversely, three infected males were mated with six uninfected females. None of the uninfected hamsters became infected after mating with an infected partner of the opposite sex, indicating that borreliosis is not sexually transmitted. These investigators failed to demonstrate contact transmission of *B. burgdorferi* between infected female - or male- hamsters and uninfected hamsters of the opposite sex. Also, it was not possible to transmit borreliosis to uninfected hamsters with urine or feces from infected hamsters.

Sadly, the observations of Middleveen et al.(1) have already generated an inordinate amount of fear and anxiety within the lay community due to sensationalized reports of its unconfirmed findings by an uncritical - and often naïve - press. This has already caused much harm, as evidenced by the fact that I have received numerous inquiries from distraught individuals, wondering if they now should even consider marrying their spouse-to-be - and risk the possibility of giving birth to an infected or congenitally deformed child - because that person had been diagnosed and treated for Lyme disease in the past.

To examine the issue of *in utero* transmission of infection, Moody and Barthold (2) inoculated pregnant female Lewis rats with viable *B. burgdorferi*, at four days of gestation. All of the inoculated pregnant females became seropositive as expected, and *B. burgdorferi* could be cultured from their spleens at 20 days of gestation; however, their placentas and fetuses were culture negative, indicting the lack of *in utero* transmission.

Moody and Barthold (2) used two different experimental protocols to determine if transplacental transmission of *B. burgdorferi* occurs. One protocol involved six non-pregnant infected females that were subsequently mated and became pregnant. Three of the females were allowed to carry to full term, whereas the remaining three were sacrificed just prior to parturition. All offspring and offspring-to-be were found to be culture negative for *B. burgdorferi*, as well as seronegative for antibody specific for *B. burgdorferi*, indicating that transplacental transmission of infection does not occur. In the second protocol, six females were infected *via* tick bite after becoming pregnant, and were allowed to carry their fetuses to birth; all were negative for infection. The results of these studies likewise failed to provide evidence for the transplacental transmission of naturally acquired borreliosis.

Other investigators examined the possibility of congenital birth defects in humans with Lyme disease by doing a rather large comparative study involving 5,000 infants, half from an area in which Lyme disease was endemic and half as controls from an area without Lyme disease (5). They found no significant differences in the overall incidence of congenital malformations between the two groups. In another study, involving 1,500 subjects including controls, no increased risk of giving birth to a child with a congenital heart defect was noted in women who had either been bitten by a tick or had been treated for Lyme disease during or before pregnancy (6). Finally, an extensive analysis of the world literature revealed "that an adverse outcome due to maternal infection with *B. burgdorferi* at any point during pregnancy in humans is at most extremely rare" (7).

I hope that my brief account of the rigorously peer-reviewed research conducted by others, will allay some of the fears and anxieties precipitated by the unconfirmed work of Middleveen et al. (1) and put this matter in proper perspective.

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Natural Killer Cell Counts Are Not Different between Patients with Post-Lyme Disease Syndrome and Controls[▽]

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It has been reported that patients with "chronic Lyme disease" have a decreased number of natural killer cells, as defined by the CD57 marker. We performed immunophenotyping in 9 individuals with post-Lyme disease syndrome, 12 who recovered from Lyme disease, and 9 healthy volunteers. The number of natural killer cells was not significantly different between the groups.

Lyme disease, the most common vector-borne illness in the United States, is caused by *Borrelia burgdorferi* and transmitted by the bite of the *Ixodes* sp. tick (the deer tick). The disease usually begins with erythema migrans, an expanding skin lesion at the site of the tick bite. Within several days or weeks, there is hematogenous dissemination of the spirochetes, and patients may present with dermatologic, neurological, cardiac, and rheumatologic involvement (7). "Chronic Lyme disease" is a controversial term applied to a broad spectrum of patients, including individuals with Lyme disease and those with post-Lyme disease syndrome (PLDS), as well as patients with no evidence of current or past *B. burgdorferi* infection (5, 6). PLDS is defined as the persistence or relapse of nonspecific symptoms (such as fatigue, musculoskeletal pain, and cognitive complaints) in patients who have had Lyme disease and have received an adequate course of antibiotic therapy.

It has been reported that patients diagnosed with chronic Lyme disease have a decreased number of natural killer cells, as defined by the CD57 marker, and that the changes in the number of $CD57^+$ cells can be monitored as evidence of response to therapy (8–10). CD57 was initially used as a marker for NK cells, but it is not expressed by all NK cells and is also expressed by T-cell subpopulations. It is thought that CD57 is a marker of terminally differentiated cells (4). Currently, the most common approach for identifying NK cells utilizes a combination of CD56 and CD16 surface markers used together with CD3 to exclude T cells expressing NK markers (NK T cells). The CD57 test is offered in some clinical laboratories and is being used by some health practitioners to evaluate and follow patients diagnosed with chronic Lyme disease. To further evaluate the utility of NK cell numbers in evaluating and/or monitoring this patient group, we performed immunophenotyping in 9 patients with PLDS, 12 individuals who recovered from Lyme disease, and 9 healthy volunteers.

Patients with PLDS had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition (1, 2), a prior positive serologic analysis confirmed by immunoglobulin G Western blotting (3), received at least one course of recommended antibiotic therapy (11), and had persistent or intermittent symptoms for at least 6 months after appropriate antibiotic therapy for Lyme disease. Common symptoms included widespread musculoskeletal pain and fatigue, memory and/or concentration impairment, and radicular pain, paresthesias, or dysesthesias. The onset of symptoms was coincident with or within 6 months of initial *B. burgdorferi* infection, symptoms were severe enough to interfere with daily life activities, and other causes were excluded. Individuals who recovered from Lyme disease also had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition and received recommended antibiotic therapy but had no complaints attributed to the disease. Controls included healthy volunteers from areas of endemicity ($n = 9$) with no previous history compatible with Lyme disease and who were seronegative for *B. burgdorferi*. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all individuals signed informed consent forms.

Peripheral blood specimens were obtained by phlebotomy on site. Anticoagulated (EDTA) samples were stained using the whole-blood lysis method and analyzed concurrently on a dual-laser FACSCalibur (BD Biosciences) using CellQuest software (BD Biosciences). Directly conjugated mouse anti-human monoclonal antibodies against CD3, CD4, CD8, CD20, CD16, CD56, and CD57 were used. Irrelevant, directly conjugated, mouse anti-human monoclonal antibodies were used to define background staining. All monoclonal antibodies were obtained from BD Biosciences and Beckman Coulter and used as recommended by the manufacturers. Lymphocytes were identified by forward and side scatter, and the lymphocyte gate was confirmed using the CD45/CD14 LeucoGate reagent (BD Biosciences). To calculate the absolute numbers of each lymphocyte subset, the percentage of positive cells was multiplied by the absolute peripheral blood lymphocyte count obtained using an automated hematology instrument on the same blood sample. Results were compared by Kruskal-Wallis test or Mann-Whitney test. The Spearman rank correlation coefficient was used to calculate quantitative correlations. All P values

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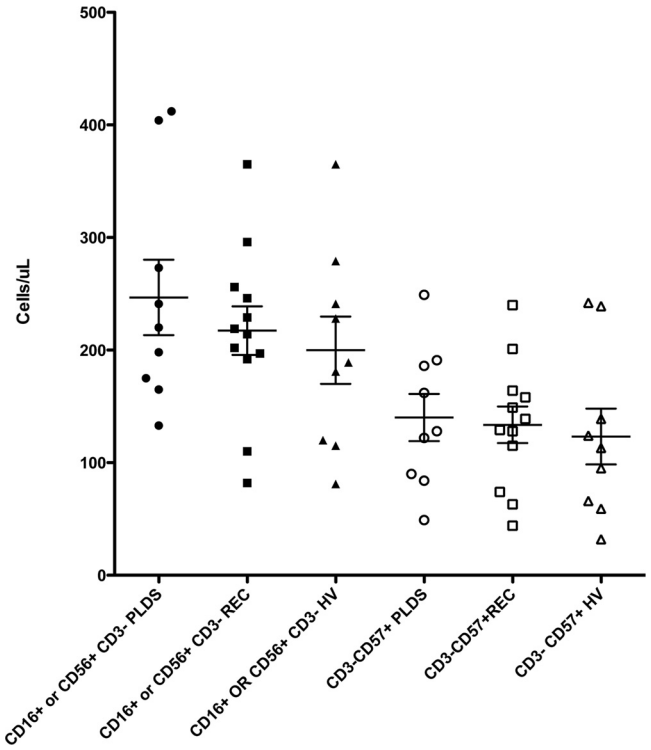

FIG. 1. Natural killer cell numbers ($CD16^+$ or $CD56^+$ $CD3^-$) and $CD3^-$ $CD57^+$ cell numbers do not differ between PLDS patients, individuals who have recovered from Lyme disease (REC), and healthy volunteers (HV).

were two sided and regarded as statistically significant if P was <0.05.

There were six women and three men in the PLDS group, six women and six men in the recovered group, and four women and five men in the healthy volunteer group. All participants were Caucasian. The median ages in the PLDS, recovered, and healthy volunteer groups were 52, 59, and 52 years, respectively. The initial presentation of the disease was a single erythema migrans lesion in two patients, a flu-like illness in one, multiple erythema migrans lesions in one, and neurological disease in five patients in the PLDS group. In the recovered group, three patients presented with a single erythema migrans lesion, four patients presented with multiple erythema migrans lesions, and five presented with neurological disease. The time that elapsed from the initial presentation of the disease to the date of lymphocyte phenotyping was longer in the PLDS group (mean, 84 months) than for the recovered group (mean, 50 months), but the difference did not reach statistical significance ($P = 0.095$).

There was no significant difference between the three groups regarding the number of $CD3^-$ $CD57^+$ ($P = 0.68$) or $CD16^+$ or $CD56^+$ $CD3^-$ cells ($P = 0.65$) (Fig. 1). There was also no difference between the groups regarding the numbers of $CD3^-$ $CD8^+$ $CD57^+$ ($P = 0.54$), $CD3^-$ $CD56^+$ $CD57^+$ ($P = 0.75$), and $CD3^-$ $CD56^-$ $CD57^+$ cells ($P = 0.13$). Very few cells were $CD3^-$ $CD56^-$ $CD57^+$, and the Spearman rank-order correlation coefficient between $CD3^-$ $CD57^+$ and $CD3^-$ $CD56^+$ $CD57^+$ cells was 0.98 ($P < 0.0001$). We conclude that the numbers of NK cells do not differ between patients with PLDS, individuals who have recovered from Lyme disease, and healthy volunteers and that the number of $CD57^+$ non-T ($CD3^-$) cells is not helpful in evaluation or management of these patients.

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MAJOR ARTICLE

A Systematic Review of *Borrelia burgdorferi* Morphologic Variants Does Not Support a Role in Chronic Lyme Disease

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Background. Much of the controversy that surrounds Lyme disease pertains to whether it produces prolonged, treatment-refractory infection, usually referred to as chronic Lyme disease. Some have proposed that round morphologic variants of *Borrelia burgdorferi*, known variably as "cyst forms" and "L-forms," are responsible for the pathogenesis of chronic Lyme disease. We have undertaken a systematic review of the literature to determine if there is a documented role of these variants in Lyme disease pathogenesis or in syndromes compatible with chronic Lyme disease.

Methods. Two systematic literature searches were performed to identify studies in which round morphologic variants of *B. burgdorferi* have been described in situ in human specimens.

Results. Our primary literature search identified 6 studies that reported round morphologic variants of *B. burgdorferi* in specimens obtained from 32 total patients. No study described these forms in patients who had purely subjective symptom complexes (eg, fatigue or pain). No study investigated a causal relationship between morphologic variants and clinical disease or evaluated treatment of morphologic variants in vivo. Of 29 additional studies that described the morphology of *B. burgdorferi* from patients with Lyme disease, the organism was invariably described as having spirochetal morphology.

Conclusions. In the context of the broader medical literature, it is not currently possible to ascribe a pathogenic role to morphologic variants of *B. burgdorferi* in either typical manifestations of Lyme disease or in other chronic disease states that are often labeled chronic Lyme disease. There is no clinical literature to justify specific treatment of *B. burgdorferi* morphologic variants.

Keywords. *Borrelia*; Lyme disease; cyst; L-form; spheroplast.

Lyme disease, which is caused by the tick-borne spirochete *Borrelia burgdorferi* sensu lato, is by far the most common vector-borne infectious disease in the temperate northern hemisphere. Many aspects of the pathogenesis, clinical manifestations, appropriate treatment, and outcomes of Lyme disease are well-accepted by the mainstream medical and scientific communities. There is considerable controversy, at least in the public discourse, about "chronic Lyme disease." This is a largely undefined term that is applied by a small minority of practicing clinicians to patients with a wide variety of presenting symptoms. Moreover, the diagnosis is not contingent upon laboratory evidence of *B. burgdorferi* infection. Most often such patients lack the objective clinical findings that are most closely associated with Lyme disease [1–10]. In contrast to authentic infection with *B. burgdorferi*, a diagnosis of chronic Lyme disease is often given to patients who either have alternative medical diagnoses or who have syndromes of prolonged, unexplained subjective complaints such as fatigue, pain, and/or cognitive dysfunction [11, 12]. Two central assumptions accompany this diagnosis: Such syndromes are caused by chronic, cryptic infection with *B. burgdorferi*; and *B. burgdorferi* assumes a

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fastidious biology in these infections that necessitates prolonged antibiotic therapy.

Advocates for greater recognition of chronic Lyme disease have presented a number of arguments meant to validate the biological plausibility of this concept. Perhaps the most commonly voiced theory contends that morphologic variants of the *B. burgdorferi* spirochete, known variably in the medical literature and lay Internet content as "L-forms," "cyst forms," "spheroplasts," "morphologic variants," "propagules," "round bodies," and "cell wall–deficient forms," are responsible for chronic Lyme disease [13–16]. In fact, articles about morphologic variants of *B. burgdorferi* constituted more than 10% of 176 publications submitted to contest practice guidelines for Lyme disease from the Infectious Diseases Society of America [17, 18]. In some cases, patients with a diagnosis of chronic Lyme disease have been treated with antibiotics believed to be selectively active against these morphologic forms, such as metronidazole and tinidazole [19, 20].

The terminology around morphologic variants of *B. burgdorferi* has proved confusing (Table 1). The commonly used terms "cyst" and "cystic" are often used colloquially to describe round morphologies of *B. burgdorferi* when seen microscopically. In microbiologically strict terms, there is no true encystment performed by this organism as is the case among a few bacterial genera, such as *Azotobacter*, *Azospirillium*, and *Rhodospirillium*. As this has become recognized, less specific descriptors such as "round bodies" have come into more common use regarding chronic Lyme disease.

We have undertaken a systematic review of the medical and the scientific literature to evaluate whether these morphologic variants of *B. burgdorferi* play a role in human Lyme disease, whether they have been associated with illnesses compatible with "chronic Lyme disease," and whether there is evidence to support antibiotic choices meant to eradicate these morphologic variants.

METHODS

Searches of the medical literature were designed to examine the evidence that "cystic" morphologic variants of *Borrelia burgdorferi* are associated with any specific form of human disease.

We performed a Boolean search of Medline (via PubMed), Embase (via OvidSP), and Thomson Reuters (formerly ISI) Web of Knowledge for studies of *B. burgdorferi* morphologic variants and their role in the microbial pathogenesis or natural history of Lyme disease. Two searches were performed. The first was intended to identify articles specifically reporting the presence of morphologic variants of *B. burgdorferi* identified in situ in human specimens. The second search was intended to evaluate more generally the description of *B. burgdorferi* in specimens from human patients with established Lyme disease.

Table 1. Terminology That Has Been Used to Describe Morphologic Variants of *Borrelia burgdorferi* and Conventional Definitions of the Terms Used

Term	Description
L-form	Bacteria with phenotypic deficiency of the rigid cell wall, usually described in the context of antibiotic exposure, noxious growth conditions, or genetic alteration. L-forms have been observed in many bacterial species, including *Borrelia burgdorferi* [21].
Alternative nomenclature	
Cell wall–deficient form	
L-variant	
L-phase	
L-organism	
Subtypes	
Stable L-forms	Cell wall alterations are permanent (ie, genetic). Stable L-forms cannot revert to parental "N-form."
Unstable L-forms	Cell wall alterations are temporarily induced by exposure to certain conditions. These include drugs (eg, penicillin). May revert to parental "N-form" once noxious conditions are removed.
Spheroplast	L-form in which some cell wall structure is retained. May be stable or unstable.
Protoplast	L-form in which no cell wall structure is retained. May be stable or unstable.
Cyst	In bacteriology, a differentiated structure that is resistant to desiccation or other noxious conditions. Cysts are characterized by a central body surrounded by a membrane-derived capsule [22, 23]. *Borrelia burgdorferi* is not known to produce cysts.
Propagule	Propagules refer to infectious "units" of material that transmit disease. These may be composed of a mix of microbial and host material.
Round, coccoid, globular, or spherical	Descriptive morphologic terms, not biologically defined.
Bleb	An irregular membrane bulge.

For the first search, our medical subject heading terms (for Medline), EMTREE terms (for Embase), and text (for others) were [(Borrelia OR Lyme) AND (cyst OR spheroplast OR "morphologic variant" OR "L-form" OR "cell wall-deficient" OR "cell wall-free" OR pleomorphic OR "round body" OR propagule)].

In addition, we reviewed the references contained in a bibliography of *B. burgdorferi* "round forms" maintained by a Lyme disease advocacy website [24]. This bibliography contained 63 references about *B. burgdorferi* and 199 references about other microorganisms, such as *Treponema pallidum*. We restricted our review to references specific for *B. burgdorferi*. A number of studies showing subcellular membrane structures, that is, "blebs," were listed in this bibliography but not retrieved in our database searches. Perusal of these articles showed that the term was mainly restricted to subcellular membrane defects observed on spirochetes, rather than ultrastructural changes in bacterial morphology. We excluded these articles because these were felt to not be synonymous with the bacterial morphologies relevant to this study.

An additional literature search was performed in Medline to identify studies describing the morphology of *B. burgdorferi* as seen in vivo in human infection. This search was performed because articles reporting morphologic variants might not actually be identified by morphology-based search terms. The additional search terms were [(Lyme OR borrelia) AND ("electron microscopy" OR "electron micrograph" OR autopsy OR histopathology OR biopsy)].

Articles were only included if they reported direct morphologic characterization of *B. burgdorferi* within a human tissue specimen. Articles (and results within articles) were excluded if they characterized morphology only after culture.

We searched the databases between inception and 10 May 2013. We also searched the reference list of each study, as well as those of relevant reviews, editorials, and correspondence that were returned in our database search. Case reports, case series, and scientific studies were included provided we could access full text in English. We excluded reviews, correspondences, expert opinions, editorials, meeting abstracts, poster presentations, and proceeding papers, as these sources lacked independent data or sufficient detail to assess the observations.

RESULTS

Search Results

Our first search yielded 57 results from Medline, 90 results from Embase, and 54 results from Thompson Reuters Web of Knowledge. From these databases 23, 26, and 20 references were selected, respectively, for further review based upon the parameters described above. After adding additional studies from the LymeInfo.net bibliography [24] and eliminating duplicates, a total of 41 studies were ultimately included in our review.

Among these 41 references were 9 relevant articles involving human subjects [15, 25–32]. In addition, there were 3 mouse studies, 28 studies done in vitro only, and 1 tick study. None of the mouse studies reported the identification of round morphologic forms of *B. burgdorferi* in vivo [33–35]. Two studies describing the effects of spirochete cultivation in ex vivo human tissue (cerebrospinal fluid and tonsillar tissue) were considered to be culture experiments rather than direct demonstration of the disease process in vivo [36, 37].

Round morphologic variants were reported in the findings of 6 of these 9 studies (Table 2) [25–28, 30, 38]. Three studies did not report morphologic results in their findings [15, 29, 32]. Altogether, these 6 "positive" studies had specimens from approximately 63 total subjects (the exact number is not possible to determine). Round *Borrelia* morphologies were described microscopically in up to 32 total patients. With the exception of a single case report from the United States, these studies and all of their subjects were from Europe.

Table 2. Characteristics of Studies Reporting Round Morphologic Variants of *Borrelia burgdorferi* in Specimens From Human Subjects

Reference	Study Subjects	Countries	No. of Subjects	Source	No. Positive[a]
[25]	Cutaneous Lyme disease[b]	Austria and Germany	43[b]	Skin biopsy	15
[26]	Erythema migrans	Bulgaria	1	Skin biopsy	1
[28]	Erythema migrans	Czech Republic	5	Skin biopsy	4
[27]	Multiple sclerosis	Norway	10	Cerebrospinal fluid sediment	8[c]
[30]	Alzheimer disease	United States (Arizona)	1	Brain	1
[38]	Alzheimer disease	Switzerland	3	Brain	3

[a] The total number of positive subjects was not made clear in 2 of the references; thus, this column represents the maximum number of positive specimens.

[b] Conditions included erythema migrans (19), prior erythema migrans (3), and acrodermatitis chronicum atrophicans (21). Subjects with a variety of other skin conditions were included in this study, making a total of 103 clinical subjects and 7 controls.

[c] This study reported 8 specimens that were positive on examination of cerebrospinal fluid sediment. Other methods performed after 4–7 months of culture were positive in all 10 subjects. These were not considered in vivo demonstrations of *B. burgdorferi* morphology.

Study Descriptions

The following are summaries of the reports describing morphologic variants of *B. burgdorferi* from human specimens.

Cutaneous Lyme Disease

A case report described a single untreated patient from Bulgaria who had presented with erythema migrans [26]. A biopsy was obtained from the skin lesion. The following findings were reported: "In the sections from the deeper strata of the dermis (str. reticulare) *Bb* [*Borrelia burgdorferi*] was observed in two different structural forms: (a) cylindrical bodies (protoplasm cylinder) with circular ends, covered with a three-layered membrane which undulated in places (Figure 2); (b) in most of the sections another structural form of the spirochete was found: granules, situated among the collagenous fibres in places closely adhered to them, sometimes covered with a membrane." The authors did not examine negative control specimens.

Another European study presented microscopic findings from 4 patients with erythema migrans [28]. Both spirochetal and "cystic" morphology were observed by light and electron microscopy. Round forms were seen primarily in dermis obtained from the central part of erythema migrans lesions; 2 healthy control specimens were negative.

A larger study reported findings from 4-mm biopsies of 103 patients with a variety of skin conditions as well as 7 control subjects [25]. The study patients included 19 patients with erythema migrans, 3 with former erythema migrans, and 21 with acrodermatitis chronicum atrophicans. Positive control slides were prepared from a *Borrelia*-injected skin model. Negative controls included normal skin sections; additionally, negative labeling controls were prepared by incubating specimens with swine serum rather than the primary antibody. *Borrelia* was immunolabeled in biopsy specimens using the antibody H9724 and visualized using videomicroscopy. Organisms were visualized in 25% of specimens. The investigators described a number of morphologic features including tangles, rope ladder–like structures, intertwined borreliae, filamentous, granules, rods, vibrio-like, a "gemma"-like body, and spheroplasts. Larger "granules" up to 3 μm were detected in areas of inflammatory infiltrates. A seronegative patient who ultimately had neuralgias 6 months later reportedly had "perineural rod-like structures," and "agglutinated intertwined spirochetes" were seen in specimens from acrodermatitis chronicum atrophicans.

Alzheimer Disease and Multiple Sclerosis

One study reported the brain pathology of a deceased patient from Arizona who had died suddenly after a short illness characterized by cognitive dysfunction [30]. The authors reported that a comprehensive workup had been done to evaluate medical causes of her syndrome, but the results of Lyme disease serologic testing and spinal fluid examination were not provided. A provisional diagnosis of Alzheimer disease was made before the patient's death, and postmortem examination of the brain was consistent with this diagnosis. The actual or presumed cause of death was not reported. According to the report, "an unexpected observation was the identification of cystic forms of the *Borrelia* spirochete in dark-field preparations of cultured hippocampus, and in imprints of hippocampus using the monoclonal antibody H9724 . . . Oil immersion examination of sections from the hippocampus impregnated with silver disclosed a rare cystic structure." Positive and negative tissue controls were stained and examined using the same methodology.

Three deceased European patients with pathologically confirmed Alzheimer disease were found to have brain tissue cultures positive for *B. burgdorferi* [31, 39]. Histopathologic examination using OspA monoclonal antibody labeling revealed a variety of structures, described as spherules, loops, rings, and cysts [38]. These varied from 4 μm to >30 μm in diameter. No antemortem clinical information was provided. The investigators also examined brains from 3 patients without neurologic disease or neuropathology as negative controls. They did not report whether blinded observations were made by additional investigators.

In a study of 10 patients with multiple sclerosis (MS), cerebrospinal fluid (CSF) sediment was examined by dark-field microscopy [27]. *Borrelia burgdorferi* "cysts" were described in 8 of these 10 specimens. No immunolabeling was performed for this preculture microscopic analysis. Polymerase chain reaction (PCR) for *B. burgdorferi* was negative in all 10 cases. Transmission electron microscopy, performed after 4–7 months' incubation, revealed "cyst-like" structures in all 10 cases. These structures were "intensely labeled" using antiborrelial serum and the monoclonal antibody H5332. The authors also looked at CSF from 5 control patients who did not have MS who had been admitted for "ischialgia." One of these subjects had also had erythema migrans, and this individual was also found to have cyst-like CSF structures.

None of the studies reported blinded observations by multiple investigators. Clinical responses to therapy and/or patient follow-up were not reported in any of the above-mentioned studies.

Descriptions of Morphologic Variants In Vivo

Table 3 summarizes the characteristics used to describe morphologic variants from each of the pertinent studies and the methods used to specifically identify these forms as *B. burgdorferi*. Immunolabeling was performed in 3 studies. In 2 cases the monoclonal antibody H9724 was used; in 1 case a polyclonal anti-*Borrelia* rabbit immunoglobulin was used in addition to the monoclonal antibody H5332. Two studies did not use any specific labeling method for the forms visualized in vivo.

Table 3. Characterization of Round Morphologic Variants of *Borrelia burgdorferi* Observed in Human Specimens

Reference	*Borrelia burgdorferi* Immunolabeling	Dimensions	Morphologic Description[a]
[25]	H9724 mAb	0.2–0.4 μm	Granules
		1–3 μm	Large granules or spherical bodies ("gemmae")
		NR	Vibrio-like forms, short rods
[26]	NR	NR	(a) Cylindrical bodies with circular ends
			(b) Granules
[28]	Polyclonal rabbit anti-*Borrelia* Ig	~0.8 μm[b]	Cyst-like
	mouse mAb H5332[c]		
[27]	NR	1–5 μm	Single cysts, cysts in clusters
[30]	H9724 mAb	NR	Rare cystic structure
[38]	OspA mAb	~4–30 μm[c]	Spherules, cysts, spirochetal loops, rings

Abbreviations: Ig, immunoglobulin; mAb, monoclonal antibody; NR, not reported or not performed.

[a] Only descriptions of round morphologies are included in this table.

[b] This was estimated based on the figures provided in the studies.

[c] Immunolabeling was performed after 4–7 months of culture, not on the primary cerebrospinal fluid sediment.

Three studies either reported or allowed estimation of cyst diameter, which ranged 25-fold from 0.2 μm to 5 μm in diameter. Investigators used a number of qualitative descriptors, including "cysts," "granules," "gemmae," "cylindrical bodies," "vibrio-like" forms, and "short rods."

Reports of *Borrelia* Morphologic Variants Using Other Search Terms

Our second literature search yielded 1917 articles. Of these, 29 reported morphologic descriptions of *B. burgdorferi* seen in situ in tissues of infected humans. Tissues reported included skin from erythema migrans and acrodermatitis chronicum atrophicans [29, 40–49]; synovial fluid, synovial tissue, or ligamentous tissue [29, 43, 50–54]; cardiac tissue [55–61]; muscle tissue [62–64]; splenic and lymphatic tissue [43, 65]; brain [66, 67]; and ocular tissue [68, 69]. In all cases the bacteria had the morphology of spirochetes. Round morphologic variants were not described in any of these studies.

Systematic Studies

No study in humans or animals systematically investigated whether a defined clinical syndrome correlates with the presence or absence of morphologic variants of *B. burgdorferi*. No study in humans or animals reported a relationship between morphologic variants of *B. burgdorferi* and either objective or subjective clinical severity. No study in humans or animals evaluated whether the long-term outcome of appropriately treated Lyme disease was related to the presence or absence of morphologic variants of *B. burgdorferi*. No study in humans or animals evaluated whether alternative treatments directed at these variants would (1) result in quantitative reduction in these organisms in vivo or (2) result in improved clinical outcomes.

DISCUSSION

One of the inherent challenges facing any scientific discussion of chronic Lyme disease is that the term itself is essentially undefined, even by its staunchest advocates [70], and most individuals who have received this label either have medically unexplained symptoms (such as chronic fatigue and/or pain) or alternative medical diagnoses [11, 12]. Several lines of argument have been offered by chronic Lyme disease advocates to support the biologic plausibility of this diagnosis: (1) Antibiotics are not effective against *B. burgdorferi* when the organism is intracellular—an untenable argument as a wide variety of intracellular infections are readily treated with the major antibiotics available for Lyme disease; (2) there is animal evidence of bacterial persistence following antibiotic treatment—yet these animals are not said to have syndromes compatible with "chronic Lyme disease," and these studies are further belied by human clinical trials showing favorable outcomes; and (3) *B. burgdorferi* assumes a fastidious, treatment-refractory "cystic" or "L-form" morphology.

Many bacterial species can assume L-form properties [21]. Their clinical significance has been debated for decades [71, 72]. L-forms of *B. burgdorferi* have been observed under laboratory conditions, and advocates for chronic Lyme disease have proposed that these forms are responsible for clinical chronicity and refractoriness to treatment. In some cases antibiotics are given specifically to eradicate these forms. In this systematic review, we investigated literature describing the presence and clinical significance of *B. burgdorferi* morphologic variants specifically obtained from human patients.

We identified a small number of studies reporting morphologic variants of *B. burgdorferi* in human tissue specimens. This

body of literature consists entirely of case reports and small case series from patients with 1 of 4 clinical conditions: erythema migrans, acrodermatitis chronicum atrophicans, Alzheimer disease, and multiple sclerosis. Round morphologic variants were specifically immunolabeled in only 3 studies, ranged greatly in size, and were described using a variety of terms. Due to discrepancies in size, terminology, and labeling, it is not clear when comparing across studies that each investigative team was actually describing the same biological phenomenon. Two of the studies used the monoclonal antibody H9724, which is known to cross-react with human antigens [73–75]. This calls into question the specificity of structures identified in this way.

Approximately 21 patients from 3 studies had round morphologic variants seen in association with erythema migrans or acrodermatitis chronicum atrophicans, well-recognized cutaneous manifestations of Lyme disease [25, 26, 28]. In the broader literature, however, organisms visualized in situ from patients with active Lyme disease (including both cutaneous and extracutaneous disease) are almost invariably described as having normal spirochetal morphology—round variants compatible with L-forms are not described [29, 40–58, 62–69]. In the end, one can do little more than acknowledge that round morphologic variants have been on rare occasion described in vivo.

Round morphologic variants were also reported in 12 patients with chronic medical conditions that are not typically attributed to Lyme disease. These comprised 4 patients with Alzheimer disease and 8 patients with MS. The information provided did not allow us to determine whether these patients had active Lyme disease or had been treated for it. Undiagnosed Lyme disease and Alzheimer disease or MS may have been coincident in these subjects, but causality cannot be concluded from these studies. Further systematic investigations of patients with Alzheimer disease have failed to demonstrate evidence of neuroborreliosis by either culture or microscopy [76–79].

As for the report of "cyst-like" structures in the CSF of MS patients, it must be noted that these subjects all tested negative by PCR for *B. burgdorferi* and that no immunolabeling was performed on the uncultivated CSF sediment. An older electron microscopy study of CSF sediment did not identify structures similar to those described by Brorson et al [80]. MS and Alzheimer disease do not share the highly specific geographic distribution of Lyme disease. Even MS, which is generally distributed in more northerly latitudes of the temperate northern hemisphere, occurs in areas where Lyme disease is either rare or nonendemic [81]. One would expect a high degree of geographic concordance if Lyme disease were responsible for a significant fraction of MS. The rarity of seroreactivity to *B. burgdorferi* despite intrathecal antibody production (oligoclonal bands) in MS makes a causal relationship with Lyme disease doubtful [82, 83].

We were unable to find even a single case report associating morphologic variants of *B. burgdorferi* with syndromes commonly diagnosed as chronic Lyme disease, such as chronic fatigue, neurocognitive dysfunction, chronic pain, or behavioral disease. Nor did we find published evidence of morphologic variants in patients with "post–Lyme disease syndromes," individuals with symptoms persisting for months after initial treatment of Lyme disease. In fact, studies of patients with post–Lyme disease syndromes have consistently failed to demonstrate the continued presence of viable *B. burgdorferi* [84–86].

The vast majority of research about *B. burgdorferi* morphologic variants has been conducted only in laboratory settings. Most of these studies are limited to describing morphology of *B. burgdorferi* in culture [36–38, 87–96]. Round morphologic variants have been shown to arise in a variety of laboratory culture conditions, including cultivation in ex vivo human tonsillar tissue and human cerebrospinal fluid. The latter 2 examples, however, cannot be assumed to approximate growth characteristics in vivo, in which the organism would face the evolving biological conditions of tissue injury and inflammation with innate and adaptive immune responses. A number of additional in vitro studies have reported that such forms arise after exposure to antibiotics or (more generally) that antibiotics induce pathologic effects on cell morphology; still others have evaluated their susceptibility to a variety of antibiotics and other compounds [97–109]. Tested compounds have included vancomycin, tigecycline, telithromycin, tinidazole, metronidazole, ranitidine bismuth sulfate, hydroxychloroquine, and grapefruit seed extract. It must be emphasized that these studies have never been performed clinically or even in animal models of Lyme disease. One can only conclude that published evidence does not justify extending such laboratory-based findings to clinical decisions for human patients.

In conclusion, there is little evidence that supports a role of *B. burgdorferi* morphologic variants in the pathogenesis of Lyme disease and no evidence that they influence treatment outcomes. The presence of round morphologic variants in vivo has been described only in a small number of case reports and case series. As different terminology and laboratory methods were used in these studies, it is difficult to be sure that in aggregate they describe similar structures. We found no convincing scientific evidence that these morphologic variants are associated with chronic *B. burgdorferi* infection, or with the sometimes disabling and protracted symptoms that are often the pretext for a chronic Lyme disease diagnosis.

Note

Potential conflicts of interest. P. G. A. has served as an expert witness in malpractice cases involving Lyme disease. G. P. W. has received research grants from the Centers for Disease Control and Prevention, the National Institutes of Health, Immunetics Inc, Bio-Rad, DiaSorin Inc, and

bioMérieux; holds equity in Abbott; has been an expert witness regarding Lyme disease in a disciplinary action for the Missouri Board of Registration for the Healing Arts and in malpractice cases involving Lyme disease; is an unpaid board member of the American Lyme Disease Foundation; and has served as a consultant to Baxter for Lyme vaccine development. P. M. L. reports no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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