LONG COVID

Symptoms after Lyme disease: What's past is prologue

Adriana Marques*

Protracted fatigue and other symptoms can occur after Lyme disease and other infections, with numerous possible drivers. Studies on posttreatment Lyme disease have been inconclusive, with no confirmed biomarker emerging. Prolonged antibiotic therapy provides no benefit. Thus, a holistic approach toward understanding and treating this complex disease is necessary.

INTRODUCTION

The question of why some patients fail to recover from an infection and continue to have unexplained fatigue and other symptoms has been long debated. Possible mechanisms, not necessarily exclusive, include factors associated with host immune response (immune dysregulation and autoimmunity), antigen or pathogen persistence, misdiagnosis, comorbidities, and psychosocial influences. The cause of persistent symptoms after antibiotic treatment of Lyme disease and the concept of chronic Lyme disease (CLD) continue to be highly controversial topics, originating from historical, social, and biological aspects of the disease (*1*–*3*).

Lyme disease, or Lyme borreliosis, is caused by spirochetes of the genus *Borreliella* (also named *Borrelia burgdorferi* sensu lato complex or Lyme borrelia) that are transmitted by the bite of infected ticks of the *Ixodes ricinus* complex. First recognized in the United States in the mid-1970s, Lyme disease is the most common vector-borne illness in the temperate zones of the Northern Hemisphere (*2*). The geographic range and number of cases continue to increase because of environmental factors, including climate change (*4*). Lyme disease in North America is caused by infection with *B. burgdorferi*, with a few cases by *B. mayonii. Ixodes scapularis* (the black-legged or deer tick) is the main vector of the infection, and *Peromyscus leucopus* (the white-footed mouse) is the key reservoir for *B. burgdorferi* in the United States. Lyme disease in Europe is predominantly caused by *B. afzelii* and *B. garinii*, followed by *B. bavariensis* and *B. burgdorferi* (*5*).

For clinical purposes, Lyme disease is divided into early localized, early disseminated, and late stages, but manifestations can overlap and appear independently. Lyme disease usually starts with erythema migrans, a distinctive skin lesion at the site of the tick bite. Systemic symptoms without erythema migrans or preceding erythema migrans can also be initial manifestations. Within days or weeks from initial infection, *Borreliella* can disseminate and establish infection at distant sites. Early disseminated manifestations can include multiple erythema migrans lesions, early Lyme neuroborreliosis, and Lyme carditis. Lyme arthritis, a later manifestation, usually occurs 6 months after the initial erythema migrans. Borrelial lymphocytoma and acrodermatitis chronica atrophicans are manifestations seen in infections acquired primarily in Europe.

Clinical variations occur depending on the infecting *Borreliella* species and virulence within the same species (*5*). Lyme arthritis is mostly associated with *B. burgdorferi*, whereas *B. garinii* and *B. bavariensis* are associated with Lyme neuroborreliosis. Borrelial lymphocytoma and acrodermatitis chronica atrophicans are primarily caused by *B. afzelii*. Systemic symptoms, fever, and multiple skin lesions are more common in patients with erythema migrans caused by *B. burgdorferi* infection acquired in the United States, compared with *B. afzelii* and *B. garinii*. Infection with *B. burgdorferi* strains classified as ribosomal spacer type (RST) 1, particularly subtype A of the outer surface protein C (OspC), is more likely to disseminate hematogenously, cause more severe early disease, and carry a higher risk of postinfectious Lyme arthritis (*6*). The mechanisms underlying these differences are not understood but relate to gene dosage and allelic variation in surface-expressed lipoproteins that help *Borreliella* evade host immune response and facilitate binding and invasion of host tissues (*7*).

The lack of a sensitive direct diagnostic test substantially contributes to the controversy surrounding Lyme disease. Direct detection of *Borreliella* in clinical samples is difficult for most clinical presentations because of the low bacterial load in human disease and preferential localization of *Borreliella* in tissues. Current laboratory tests are based on detection of antibody responses in serum. Serodiagnostics approaches work relatively well if used as recommended, and the modified two-tier testing algorithm has improved sensitivity in early Lyme disease. Still, because of the window period, patients early in their illness can have negative test results. These patients should receive treatment on the basis of clinical diagnosis. Interpretation of serologic results can be confusing to health care providers and patients, and false-positive results can occur, particularly with immunoglobulin M (IgM)–based tests. Importantly, current assays do not distinguish between active and inactive infection and cannot be used to monitor response to therapy. This complicates serodiagnosis of reinfections and can cause confusion for patients. The use of nonvalidated Lyme diagnostic tests is problematic and not recommended (*8*).

SYMPTOMS AFTER TREATMENT OF LYME DISEASE

Most patients fully recover after treatment with recommended antibiotic regimens. Patients with later manifestations can take longer to recover, and some damage can be nonreversible, as seen in patients with residual weakness due to facial nerve palsy. Treatment failure, with objective signs of disease after treatment, is uncommon. Postinfectious Lyme arthritis, or antibiotic-refractory

Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-1888 USA.
*Corresponding author. Email: amarques@niaid.nih.gov

Lyme arthritis, is a complication that occurs in about 10% of patients with Lyme arthritis (*9*). These patients continue to have inflammatory proliferative synovitis despite 2 to 3 months of antibiotic therapy and are treated with disease-modifying antirheumatic drugs. Host genetic factors, such as Toll-like receptor 1 (*TLR1*) gene polymorphism and certain human leukocyte antigen (*HLA*)–*DR* alleles, together with *B. burgdorferi* virulence factors, such as infection with *B. burgdorferi* RST1 OspC type A strains, are associated with postinfectious Lyme arthritis (*6*). Infection results in an excessive proinflammatory immune response that continues into the postinfectious period with high amounts of interferon-γ (IFN-γ), increased T helper 1 (T_H1) and T_H17 immune responses, and autoreactive T and B cells. *B. burgdorferi* peptidoglycan has been detected in synovial fluid from patients with postinfectious Lyme arthritis and likely has a role in maintaining inflammation (*10*).

Posttreatment Lyme disease symptoms or syndrome (PTLDS) resembles other postinfectious fatigue syndromes. These individuals have unexplained persistent or relapsing nonspecific symptoms starting within 6 months after treatment of a documented episode of Lyme disease (*1*). The most common complaints are fatigue, difficulties with concentration and memory, myalgias, arthralgias, depression, anxiety, paresthesias, and sleep problems. In comparison with another post infectious condition, Long Covid, shortness of breath, changes in smell or taste, palpitations, chest pain, and cough are not reported in PTLDS. The prevalence of subjective complaints 6 to 12 months after treatment ranges from 0 to 27% in prospective studies of patients with erythema migrans (*11*–*37*). This is similar to the estimated prevalence of Long Covid, ranging from 0.3 to 2% (*38*), 6.6 to 10.3% (*39*), and 12.7% (*40*), and to the prevalence of persistent fatigue or pain after other infections.

POSSIBLE CAUSES OF PTLDS

Risk factors associated with PTLDS resemble other postinfectious syndromes, providing clues to components contributing to persistent symptoms. Factors related to the infection include more severe disease, as marked by presentation with multiple erythema migrans or disseminated manifestations, higher symptom severity at the start of treatment, the presence of tingling or an abnormal skin sensation at the initial visit, higher bacterial load in skin, and antibiotic treatment delay. Other risk factors include older age, female sex, presence of comorbidities, poor social and physical functioning, history of stressful events, more negative perceptions of illness, preexisting anxiety or depression, and higher anxiety and depression scores at the start of antibiotic treatment (*26*, *27*, *30*, *32*, *41*–*44*). Most likely, several factors contribute to predisposing and perpetuating these symptoms in an individual patient (Fig. 1).

Convalescence, misdiagnosis, and other conditions

Most patients continue to improve after treatment for Lyme disease, with fewer patients reporting symptoms over time. This is an expected convalescent period, as seen with other infections, and an important point to emphasize and reassure worried patients. Moreover, symptoms and signs due to other conditions can be wrongly attributed to Lyme disease, with an expected poorer response to treatment. For example, patients with a definite diagnosis of Lyme neuroborreliosis have better outcomes after treatment when compared with possible cases (*45*). Similarly, nonspecific symptoms can be caused by preexisting or new conditions unrelated to Lyme disease. The presence of comorbidities is associated with long-term symptoms and lower quality of life scores in Lyme disease (*42*), and it can be difficult to attribute or exclude a nonspecific symptom to a specific condition. Of note, coinfections with other tick-borne pathogens are rare in PTLDS (*41*, *46*–*48*).

Common factors

Nonspecific symptoms like fatigue, chronic pain, and sleep disturbances are common in the general population. Prospective studies examining patients with Lyme disease and controls showed more than 20% prevalence of these symptoms in controls. Five prospective studies showed a similar or lower prevalence of symptoms in patients with early Lyme disease (*22*, *33*, *34*, *36*, *37*), although two studies showed higher prevalence of symptoms in patients (*27*, *35*) when compared with that in controls. The LymeProspect cohort study, conducted in The Netherlands, is particularly interesting (*35*). This study compared the prevalence of persistent fatigue, cognitive impairment, and pain between cohorts of 1135 patients with Lyme disease, 2405 individuals with a tick bite, and a general population cohort of 4000 individuals. The prevalence of symptoms at the 1-year follow-up was 27.2% in the Lyme disease cohort, 23.3% in the tick bite cohort, and 21.2% in the general population cohort. An analysis of baseline factors associated with persistent symptoms at 1 year found that the main predictors were similar between patients and controls (*41*). These predictors included poorer physical and social functioning; higher depression and anxiety scores; more negative illness perceptions; comorbidities; and more severe fatigue, cognitive impairment, and pain. The cognitive-behavioral responses to symptoms also contributed to the prediction of symptom persistence. A Lyme disease diagnosis was only moderately associated with the presence of symptoms. The importance of an individual's positive or negative expectancies on symptom course and treatment outcome has also been shown in the Persistent Lyme Empiric Antibiotic Study Europe trial (*49*). Predictors of symptom improvement at both the end of treatment and at follow-up were higher pretreatment functioning, higher pretreatment expectancy to improve, and thinking that one had received antibiotics at the end of therapy (*50*). Previous exposure to traumatic life events was associated with a higher risk of PTLDS in patients with early Lyme disease (*27*). These results align with studies researching common predisposing and associated factors of complex, multidimensional symptoms like fatigue and pain. Knowledge about these factors can help identify patients at risk and develop interventions to prevent, reduce, and help manage these symptoms. How to control for these variables and integrate them into therapeutic research is a challenging topic.

Pathogen and host factors

The local early immune response and the interplay with bacterial virulence factors are important in controlling the infection and potentially affect host response, disease manifestations, and outcome. Patients with erythema migrans who had a positive culture by skin biopsy were less likely to fully recover than patients with a negative culture (*30*, *51*). Skin biopsy culture positivity is associated with bacterial load and *Borreliella* burden in the skin (*30*, *52*). Prior work showed that the bacterial load was nearly three times higher in RST1 compared with that in RST2 and RST3 *B. burgdorferi* (*52*) and that RST1 strains are more frequently associated with disseminated infection and with postinfectious Lyme arthritis. There is no evidence linking differences in *Borreliella* virulence and PTLDS, but this likely reflects difficulties

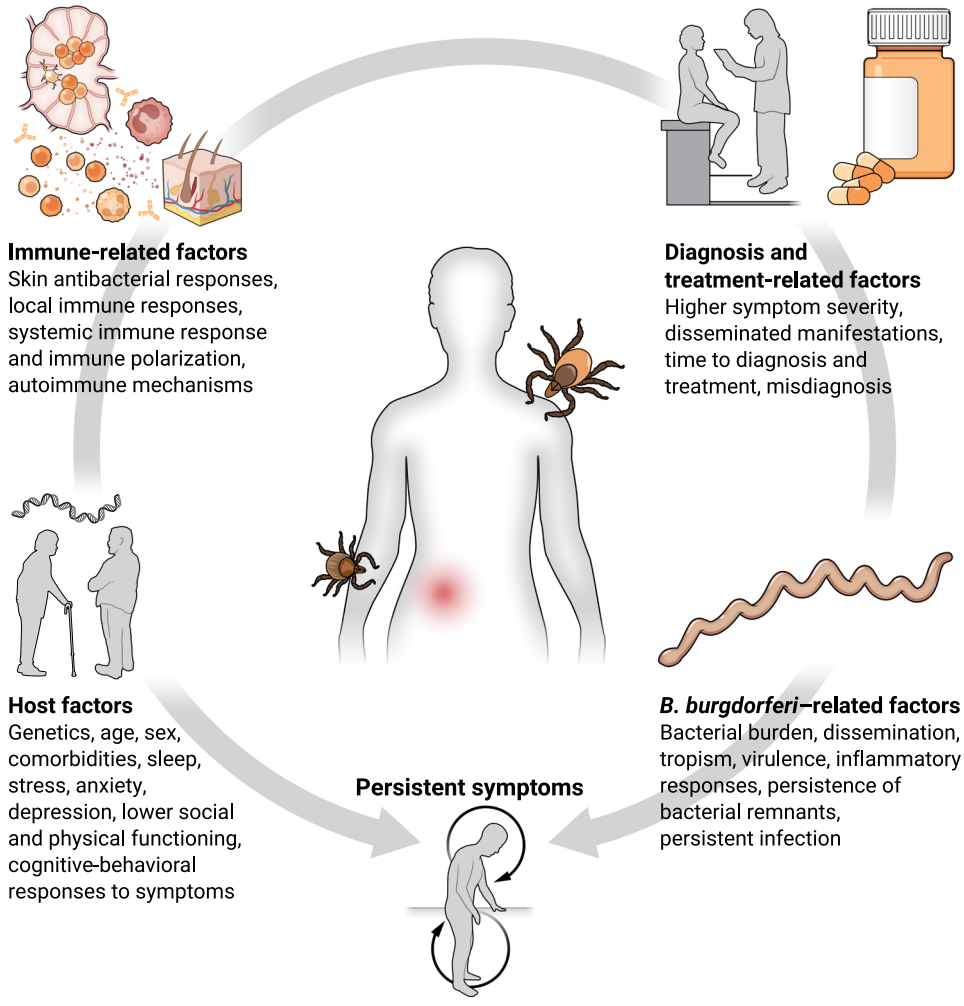

Fig. 1. Potential factors contributing to PTLDS. Several factors may interact to predispose to, or protect against, PTLDS. These include factors contributing to the effectiveness of the host immune response against the pathogen and potentially dysregulated immune responses. These may be intrinsic (genetics, age, sex, and comorbidities) and extrinsic (psychological stress, disturbed sleep, and poor health behaviors) to the host. Host factors interact with *B. burgdorferi*–related factors, which influence pathogen burden, inflammatory responses, bacterial dissemination, and tissue tropism. Delays in diagnosis and the start of antibiotic treatment influence pathogen control, disease presentation, and host responses. Misdiagnosis and other conditions can cause delays in seeking adequate care. Importantly, how an individual experiences illness will play a role in recovery.

in obtaining direct pathogen information from most patients.

Host genetic factors influence the skin antibacterial response to *B. burgdorferi*, as well as innate and adaptive immune responses against the bacteria, and are associated with susceptibility to Lyme disease, more severe presentations, and maladaptive immune responses (*6*, *53*–*55*). A lower expression of IFN-γ in erythema migrans skin biopsies was associated with PTLDS, supporting the hypothesis that a local T_H1 response is important early in the infection (*56*). Patients who returned to health had a higher number of plasmablasts at the time of diagnosis than individuals who developed PTLDS, suggesting that initial activation of B cells may influence recovery, but larger studies are needed (*57*). A missense variant at the gene encoding for secretoglobin family 1D member 2 (*SCGB1D2*), a protein expressed primarily in the skin and sweat glands, was linked to an increased susceptibility to Lyme disease, possibly because of less efficient inhibition of the bacteria in the skin (*53*). Increased Lyme disease susceptibility was also found to be associated with the rs1061632 major allele, possibly related to up-regulation of the Akt–mammalian target of rapamycin (mTOR) pathway and interleukin-10 (IL-10) responses, along with reduced specific antibody production, which may have a negative impact on bacterial clearance (*55*). A study using samples from the LymeProspect cohort (*35*) showed that cytokine responses were associated with age and sex, as well as Lyme disease manifestation and antibody production. Moreover, the study identified 34 genetic loci associated with cytokine responses, including *TLR1-6-10*, *IL6*, *IL10*, and *Klotho*, a gene encoding a protein linked to aging and age-related illnesses (*58*). The *TLR1-6-10* locus had the strongest association, with fine mapping linking to rs5743618-A. Also part of the LymeProspect study, IL-1β, IL-6, IL-10, and IL-1 receptor antagonist stimulation responses ex vivo, as well as 13 single-nucleotide polymorphisms in seven genes, were examined as predictors of persistent symptoms. Lower IL-10 concentrations at

6 weeks were associated with persistent symptoms in the primary analysis, and polymorphisms in mannose binding lectin and IL-1β were associated with attribution of persistent symptoms to Lyme disease in a subgroup analysis (*41*).

Older age is a risk factor for PTLDS, and younger patients with early Lyme disease were more likely to have a complete response (*26*). A prior study demonstrated that age impaired IFN-γ and IL-22 production in peripheral mononuclear cells stimulated with *B. burgdorferi* (*59*). Older patients have more comorbidities, which can confound symptom assessment and may increase vulnerability. Certain comorbidities may interfere with host response to Lyme borrelia and predispose to persistence of symptoms. For example, in one murine study, diet-induced obesity led to more severe pathology and inhibition of innate immune response to *B. burgdorferi* (*60*); in another mouse study, the authors found that poor sleep delayed arthritis resolution, possibly by altering host immune response (*61*). Poor and insufficient sleep correlates with fatigue, pain, anxiety, and cognitive complaints in humans, and it is an important generic factor implicated in predisposing and perpetuating fatigue due to many conditions. It also can be targeted for intervention (*62*–*64*). Female sex is a risk factor for PTLDS, as it is for most postinfectious fatigue syndromes as well as fatigue due to other conditions. Potential contributory factors are multifold and may relate to both psychosocial and biological factors (*65*).

Autoimmunity and immune dysregulation in PTLDS

The role of infection-induced autoimmunity and immune dysregulation in PTLDS has been investigated, but no reproducible marker has been identified. The concentrations of C-reactive protein, a proinflammatory marker, were shown to be higher in patients with PTLDS than those in recovered patients, suggesting that inflammation could play a role in some individuals (*66*). Other studies found that individuals with PTLDS had higher titers of antibodies to neural proteins (*67*, *68*). Antibody titers against endothelial cell growth factor were higher in one study (*69*) but not confirmed in another (*70*). Median concentrations of chemokine (C-C motif) ligand 19 (CCL19) were found to remain elevated at posttreatment visits in individuals with PTLDS, possibly reflecting an ongoing immune-driven process (*71*). These findings were not replicated in a European study, which instead found an association with persistent symptoms and higher IL-23 concentrations, suggesting the possibility that a T_H17 response could play a role in persistent symptoms (*69*). A T_H17-associated immune response has been linked to autoantibody production in postinfectious Lyme arthritis (*72*, *73*) and slower recovery in Lyme neuroborreliosis (*74*, *75*). Two studies have implicated IFN-α in PTLDS, with a recent study in patients with Lyme neuroborreliosis showing higher IFN-α concentrations in sera at the initial and follow-up visits in individuals with PTLDS when compared with those in recovered individuals and that IFN-α concentrations correlated with severity of symptoms (*76*, *77*). This is an interesting link because fatigue, myalgia, and cognitive dysfunction are known side effects of IFN-α treatment.

Transcriptomic responses to Lyme disease

The longitudinal transcriptomic response in blood has been investigated in a series of studies, but again no clear markers have been found to inform PTLDS pathogenesis. The first study found that transcriptomic changes due to the acute infection (erythema migrans) did not return to baseline at 6 months after treatment, but few differences were observed between recovered patients and patients with persistent symptoms (*78*). These sustained transcriptomic changes were not found in a study of patients with early disseminated infection (*79*). A distinct gene expression signature was seen during acute disease and early convalescence but resolved by 6 months after treatment. This study identified a 20-gene classifier that could distinguish patients with acute Lyme disease from controls. A third study demonstrated that gene expression profiles of patients with erythema migrans clustered separately from controls, with most patients in clusters 0 and 1 and most controls in cluster 2 (*80*). Patients in cluster 0 had more symptomatic acute disease, whereas patients in clusters 1 and 2 were more likely to have received initial antibiotic therapy. However, machine learning classifiers could not clearly distinguish which patients would develop PTLDS. Most cases stayed separate from controls over time (6 and 12 months), but a batch effect is possible in this study. The same group investigated the transcriptional profile of patients with PTLDS compared with that of patients with acute Lyme disease and controls using the data from the previous study (*81*). A classifier with 35 genes specific to Lyme disease could distinguish individuals with acute Lyme disease, individuals with PTLDS, and healthy controls. Because the groups were processed separately, the division between PTLDS and the other groups may be explained by batch effects. Recently, a 31-gene Lyme disease classifier was developed using transcriptomic data from patients with early Lyme disease, controls, and patients with other infections (*82*). The potential of this classifier to evaluate patients with the diagnosis of Lyme disease and symptoms after therapy requires further study. Of note, there is no overlap when comparing the genes selected for each of the three classifiers distinguishing acute Lyme disease from controls.

Persistence of *B. burgdorferi* or its remnants

One possibility is that remnants of *B. burgdorferi* could persist in tissues after antibiotic treatment, driving a persistent host response in some patients. *B. burgdorferi* peptidoglycan was found in synovial fluid of patients with postinfectious Lyme arthritis (*10*), but no studies in PTLDS have been performed to date. The questions of whether there are residual live *Borreliella* after antibiotic treatment and whether that could be driving PTLDS have been a major concern. In animal studies, low amounts of *B. burgdorferi* DNA and mRNA can be detected in tissues for months after antibiotic treatment, and *B. burgdorferi* DNA has been detected by xenodiagnosis (the use of a vector to detect the presence of an organism) in some studies (*83*–*89*). However, the bacteria could not be recovered by culture. A clinical study using xenodiagnosis with laboratory-reared *I. scapularis* larval ticks was safe but resulted in a positive test in only 1 of 17 patients with PTLDS or high titers of antibodies to *B. burgdorferi* C6 peptide (*90*). The importance of detecting *B. burgdorferi*'s DNA and whether it represents viable *B. burgdorferi* or remnants of the bacteria is a point of ongoing discussion (*91*).

There have been five randomized, placebo-controlled, double-blind clinical trials addressing the question of whether additional antibiotic treatment benefits patients with PTLDS or symptoms attributed to Lyme disease. Regimens tested against matching intravenous and oral placebos included ceftriaxone for 30 days followed by doxycycline for 60 days (*46*), ceftriaxone for 28 days (*92*), and ceftriaxone for 10 weeks (*47*). One study had an open-label period of ceftriaxone for

2 weeks, followed by random assignment of patients to 12 weeks of oral doxycycline, clarithromycin-hydroxychloroquine, or placebo (*49*). The results of these trials showed that prolonged antibiotic treatment had no lasting benefit while having potential serious risks. The studies also showed a placebo effect and that the intensity of symptoms varied over time. Additional studies of antibiotic treatment of PTLDS will require discovery of biomarkers that identify distinct subgroups and provide a strong rationale for new interventional trials. In addition, therapeutic interventional studies in PTLDS must have a double-blind, randomized controlled design.

A NOTE ABOUT CLD

The controversy surrounding Lyme disease dates to the early investigations of the disease, with differences about how Lyme disease is defined (and therefore diagnosed and treated), leading to the CLD debate that continues to this day. CLD is a contentious term, with no clear diagnostic criteria. For example, one definition is a "multisystem illness with a wide range of symptoms or signs that are either continuously or intermittently present for a minimum of 6 months." CLD has evolved into a "multiple chronic infectious disease syndrome," and patients are diagnosed with numerous infections and poorly defined conditions. Patients are often charged high fees for diagnosis and care and may spend money on unproven tests and treatments that range from harmless to dangerous. Other medical conditions may be delayed in diagnosis and treatment or can be missed altogether. Patients with PTLDS are a small subset of this large and heterogeneous group. Most patients with CLD have other conditions or are patients with persistent physical symptoms (*93*–*102*). Because of the lack of a consensus definition for CLD or on the myriad of treatments used, it is not possible to make any conclusive statements about the condition(s), but there is a critical need for high-quality research to better understand and help this diverse group of individuals.

CONCLUSION

Unfortunately, the questions about the underlying causes of PTLDS remain mostly unanswered. Studies have been inconclusive with findings that have not been replicated. The reasons are multifold, including heterogeneous populations, differences in methodology, and multiple tested variables combined with a small number of patients suffering from PTLDS in prospective studies. Currently, there are no confirmed biomarkers to help differentiate between subgroups of patients, which are necessary to help personalize care for patients suffering from prolonged symptoms. A holistic approach encompassing all aspects of the illness experience can help reduce and manage complex symptoms, and associated factors should not be overlooked. Lyme disease is a challenging area for research, and there is a critical need for more investment in fundamental, translational, and clinical research. A supported clinical network to facilitate high-quality clinical studies in all different stages of Lyme disease can help bring evidence-based answers to these open questions and improve the care of patients. Moreover, a comprehensive multidimensional model addressing common factors predisposing individuals to persistent symptoms after infections and other triggers, combining expertise across different fields, can lead to new ways to prevent, treat, and manage these conditions.

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