Determinants of persistent symptoms after treatment for Lyme borreliosis: a prospective observational cohort study

Hedwig D. Vrijmoeth,[a,h] Jeanine Ursinus,[b,c,h] Margriet G. Harms,[d] Anna D. Tulen,[d] M. E. Baarsma,[b,c] Freek R. van de Schoor,[a] Stefanie A. Gauw,[b,c] Tizza P. Zomer,[f] Yolande M. Vermeeren,[f] José A. Ferreira,[d] Hein Sprong,[d] Kristin Kremer,[d,g] Hans Knoop,[e] Leo A. B. Joosten,[a] Bart Jan Kullberg,[a,i] Joppe W. Hovius,[b,c,i] and Cees C. van den Wijngaard[d,*,i]

[a]Department of Internal Medicine and Radboudumc Center for Infectious Diseases (RCI), Radboud University Medical Center, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands
[b]Department of Internal Medicine, Division of Infectious Diseases, Amsterdam UMC, Location AMC, University of Amsterdam, P.O. Box 22660, 1100 DD, Amsterdam, the Netherlands
[c]Amsterdam Institute for Infection and Immunity, Amsterdam, the Netherlands
[d]National Institute for Public Health and Environment (RIVM), Center for Infectious Disease Control, P.O. Box 1, 3720 BA, Bilthoven, the Netherlands
[e]Department of Medical Psychology, Amsterdam UMC, University of Amsterdam, Amsterdam Public Health Research Institute, P.O. Box 22660, 1100 DD, Amsterdam, the Netherlands
[f]Lyme Center Apeldoorn, Gelre Hospital, P.O. Box 9014, 7300 DS, Apeldoorn, the Netherlands

eBioMedicine
2023;98: 104825
Published Online 27 November 2023
https://doi.org/10.1016/j.ebiom.2023.104825

Summary

Background Patients treated for Lyme borreliosis (LB) frequently report persistent symptoms. Little is known about risk factors and etiology.

Methods In a prospective observational cohort study with a follow-up of one year, we assessed a range of microbiological, immunological, genetic, clinical, functional, epidemiological, psychosocial and cognitive-behavioral variables as determinants of persistent symptoms after treatment for LB. Between 2015 and 2018 we included 1135 physician-confirmed LB patients at initiation of antibiotic therapy, through clinical LB centers and online self-registration. Two reference cohorts of individuals without LB (n = 4000 and n = 2405) served as a control. Prediction analyses and association studies were used to identify determinants, as collected from online questionnaires (three-monthly) and laboratory tests (twice).

Findings Main predictors of persistent symptoms were baseline poorer physical and social functioning, higher depression and anxiety scores, more negative illness perceptions, comorbidity, as well as fatigue, cognitive impairment, and pain in 295 patients with persistent symptoms. The primary prediction model correctly indicated persistent symptoms in 71.0% of predictions (AUC 0.79). In patients with symptoms at baseline, cognitive-behavioral responses to symptoms predicted symptom persistence. Of various microbiological, immunological and genetic factors, only lower IL-10 concentrations in *ex vivo* stimulation experiments were associated with persistent symptoms. Clinical LB characteristics did not contribute to the prediction of persistent symptoms.

Interpretation Determinants of persistent symptoms after LB were mainly generic, including baseline functioning, symptoms and cognitive-behavioral responses. A potential role of host immune responses remains to be investigated.

Funding Netherlands Organisation for Health Research and Development (ZonMw); the Dutch Ministry of Health, Welfare and Sport (VWS).

Keywords: Lyme borreliosis; Erythema migrans; Persistent symptoms

*Corresponding author. Institute for Public Health and Environment (RIVM), Center for Infectious Disease Control, P.O. Box 1, 3720 BA, Bilthoven, the Netherlands.
E-mail address: kees.van.den.wijngaard@rivm.nl (C.C. van den Wijngaard).
[g]Current address: KNCV Tuberculosis Foundation, P.O. Box 146, 2501 CC, Den Haag, the Netherlands.
[h]Authors contributed equally to this work.
[i]Shared senior authorship.

Research in context

Evidence before this study

We searched PubMed at the initiation of the study (2014) and during data analysis (January 2019), using the following search terms: "Borrelia Infections", "Borrelia", "borrelia", "borreliosis", "Erythema Chronicum Migrans", "Erythema Migrans", "lyme", "Lymes", "Lyme's", "Neuroborreliosis". We repeated this search during the writing process, reviewed all studies on Lyme borreliosis (LB) and persistent symptoms, and found several studies reporting identification of factors associated with persistent symptoms. Clinical and psychological aspects, such as manifestation, duration and number of symptoms at baseline, as well as anxiety and depression have frequently been assessed, with divergent results. This was also true for studies on persistent infection after LB or (co)-infection with other tick-borne pathogens. Reports on immunological or genetic parameters have focused on a number of cytokines, chemokines or polymorphisms. Study characteristics differed: from retrospective to prospective, and short (six months) to long-term (>10 years) follow-up, often with limited power, or without taking into account the substantial background prevalence of symptoms. The LymeProspect study was designed to prospectively explore a wide range of potential determinants of persistent symptoms, based on all hypotheses and findings that had been reported before.

Added value of this study

In a large prospective cohort of LB patients, prevalence of persistent symptoms was assessed during a one-year follow-up. Comprehensive questionnaires and sequential blood samples were collected to study potential microbiological, immunological, genetic, clinical, epidemiological, psychological, functional, and cognitive-behavioral determinants, and data were compared to the same longitudinal assessments in two reference cohorts. This broad, multi-variable approach resulted in identification of several generic determinants of persistent symptoms, including baseline functioning, symptoms and cognitive-behavioral responses to symptoms, most likely due to the substantial background prevalence of such symptoms in the reference cohorts. No evidence was found for microbiological or LB-specific clinical determinants of persistent symptoms, but an association of host immune responses with persistent symptoms was observed.

Implications of all the available evidence

Determinants of persistent symptoms after treatment for LB are predominantly general, corresponding to determinants of persistent symptoms in reference cohorts. Findings also underscore the importance of further prospective studies on the course of immunological responses and their genetic bases in relation to tick bites, acute LB and persistent symptoms after antibiotic treatment.

Introduction

Lyme borreliosis (LB) is an emerging infectious disease caused by the tick-borne spirochete *Borrelia burgdorferi* sensu lato (s.l.). After antimicrobial therapy, some patients report long-lasting symptoms, such as fatigue, cognitive impairment, and pain. This complex of symptoms, that may persist for months to years, is often referred to as Post-Treatment Lyme Disease Syndrome (PTLDS), and may substantially impact quality of life.[1–3] We documented a 27.2% prevalence of persistent symptoms in a prospective cohort of patients treated for confirmed LB during one year of follow-up. This prevalence was significantly higher than in two reference cohorts, although the prevalence of persistent symptoms in these reference cohorts was substantial (21.2% and 23.3%).[4]

Little is known about risk factors for or pathogenesis of persistent symptoms after LB. Associations with clinical and patient characteristics, including age,[5] sex,[6] and duration and number of symptoms at start of treatment[7,8] have been suggested. Microbiological and immunological hypotheses exist, including the burden of borrelial infection,[9] dysregulated immune responses,[10] persistent infection,[11] or (co-)infection with other tick-borne pathogens.[12] Genetic variation, either related to immunological responses or not, could play a role in disease outcome as well.[13,14] Furthermore, psychosocial and cognitive-behavioral aspects may be involved, including psychiatric comorbidity,[15] and patients' beliefs and behavior in response to symptoms contributing to persistence of infection-triggered symptoms, a model that has been applied to explain persistent symptoms in other conditions.[16] Since long-lasting symptoms have been described after other infectious diseases, such as Epstein–Barr virus, Q-fever and COVID-19, existence of a general post-infectious syndrome or shared underlying mechanisms for post-infectious persistent symptoms could also be argued.[17–19]

After having determined the prevalence of persistent symptoms after treatment for LB in a large prospective study in the Netherlands, in the current manuscript we explored a broad range of pre-defined potential determinants of persistent symptoms in those patients after treatment for physician-confirmed LB.[4,20] Multivariable prediction analyses and association studies were carried out with data on LB patients and data on two reference cohorts of individuals without LB.

Methods

Study design

The LymeProspect study is a prospective, observational cohort study of LB patients with a follow-up of one year.

We consecutively included 1076 adults with erythema migrans (EM) and 59 with disseminated LB between April 2015 and October 2018. Patients with a new diagnosis of physician-confirmed LB were included through self-registration online or through the participating clinical LB centers by the researchers involved (HDV, JU, SAG, MEB, FRvdS, TPZ), either before or within seven days after initiation of antibiotic therapy. Inclusion and exclusion criteria are shown in Table S1, and were verified with the threating physician. Participating clinical LB centers were Amsterdam UMC (Amsterdam, the Netherlands), Gelre hospitals (Apeldoorn, the Netherlands), and Radboudumc (Nijmegen, the Netherlands), and patients visiting these clinical LB centers because of a suspicion of LB were approached for participation by the clinical researchers. At baseline and every three months thereafter, patients completed a comprehensive set of online questionnaires consisting of standardised and validated questionnaire systems that are considered best-of-class in this area of research, as listed in Table 1 and Table S2. Blood was collected at baseline and six weeks thereafter. In a subset of patients with EM or acrodermatitis chronic atrophicans (ACA), baseline skin biopsies were taken. In addition, we included a cohort of patients who already had chronic (≥six months) symptoms of unknown origin that were attributed to LB without clinical or laboratory confirmation, to assess the presence of determinants of persistent symptoms as identified in the prospective confirmed LB cohort. Major amendments to the study design during the study were: extension of the maximum duration of pre-inclusion antibiotic therapy by EM patients at online inclusion from zero to four days, collection of skin biopsies in all LB skin manifestations (not only EM), both from June 2015 onwards; online inclusion of patients with disseminated LB manifestations (not only through clinical LB centers), and inclusion of EM patients through clinical LB centers at a maximum of seven days after start of antibiotic treatment, similar to online inclusion and to patients with disseminated LB (from December 2015).

Outcome measures

Primary outcomes of this study were determinants of persistent symptoms, as identified by prediction analyses and association studies including all variables assessed (Table S2). Persistent symptoms were defined as scoring in the impaired range on one or more questionnaires for fatigue (Checklist Individual Strength (CIS), subscale fatigue, score ≥35), cognitive impairment (Cognitive Failures Questionnaire (CFQ), score ≥44), or pain (RAND SF-36 Health Status Inventory (SF-36), subscale bodily pain, score ≤55) during at least six months, with onset within six months after initial antibiotic treatment, aligning with the PTLDS definition.[1,4] Potential determinants were derived from

Microbiological	*B. burgdorferi* s.l. serology Serology 'other' tick-borne pathogens (*Anaplasma phagocytophilum, Babesia microti, B. miyamotoi, Candidatus Neoehrlichia mikurensis, Rickettsia conorii,* Tick-borne encephalitis virus) Whole blood qPCR *Rickettsia helvetica* Skin biopsy culture Skin biopsy qPCR
Immunological	Cytokine concentrations (IL-1β, IL-6, IL-10, IL-Ra) upon *ex vivo* PBMC stimulations Cytokine concentrations (IL-1β, IL-6, IL-10) upon *ex vivo* whole blood stimulations Experimental diagnostic test results (Lyme iSpot, LTT-MELISA, SpiroFind)[19]
Genetic	Genotypes for 13 polymorphisms described in literature to have a role in *B. burgdorferi* s.l. host immune response or persistent symptoms
Clinical, functional, epidemiological and psychosocial	LB manifestation, EM size and central clearing, duration of symptoms at start of treatment, previous LB, tick bite history, antibiotic treatment, comorbidity, healthcare consumption, baseline symptoms (including PHQ-15), medication use, daily occupation and absenteeism from work Region Anxiety and depression scores, pre-existent anxiety and depression, physical and social functioning
Cognitive-behavioral	Brief-Illness perception questionnaire (B-IPQ), Cognitive and Behavioral Responses Questionnaire (CBRSQ), Self-Efficacy Scale fatigue (SES-F), Pain Catastrophizing Scale (PCS) Attribution of symptoms to LB International Physical Activity Questionnaire (IPAQ)
Potential confounders/other	Age Sex Educational level Number of baseline comorbidities Number of completed time points Laboratory processing variables

Summary of all variables considered in the multivariable and univariable analyses. Detailed overview and description are provided in Table S2. Most of the variables were assessed at baseline; microbiological and immunological also after six weeks. Some variables were only available for a subgroup of LB patients. Age, sex, educational level and comorbidity were predefined as potential confounders for association studies, and were included as potential determinants in random forest prediction analyses. Abbreviations: EM = erythema migrans, IL = interleukin, LB = Lyme borreliosis, LTT-MELISA = Lymphocyte Transformation Test - Memory Lymphocyte Immunostimulation Assay, PBMC = peripheral blood mononuclear cell, PHQ-15 = Patient Health Questionnaire, qPCR = quantitative polymerase chain reaction, s.l. = sensu lato.

Table 1: **Overview of variables considered in the prediction analyses and association studies.**

questionnaires and laboratory measurements as specified below. Additional analyses were performed to assess robustness of, and to elaborate on the primary outcomes (Table S3).

Reference cohorts

As with the data obtained in LB patients, a subset of the longitudinal questionnaire variables was available for two reference cohorts of individuals without LB, for whom inclusion criteria have been published.[4] One cohort (population cohort) comprised participants who were randomly selected from the general population database and were frequency matched (age, sex, municipality, month of inclusion) to the LB cohort. The other included individuals who had reported a tick bite online, without evidence for LB during follow-up (tick bite cohort). The recruitment and inclusion methods of this tick bite cohort were similar to patients treated for physician-confirmed LB. Subjects in the tick bite cohort with subsequent evidence or suspicion of LB or other tick-borne disease at baseline or during follow-up were excluded. Part of the laboratory tests performed in the LB cohort was also available in 190 individuals from the population cohort.[21]

Measurements

Table 1 and Table S2 provide an overview of all microbiological, immunological, genetic, clinical, functional, epidemiological, psychosocial, and cognitive-behavioral variables assessed. At baseline, demographical characteristics including gender, age, and highest educational level, as well as information on comorbidities (adapted from the Treatment Inventory of Costs in Patients with psychiatric disorders, TIC-P),[22] pre-existent symptoms (adapted from PREDIS),[23] and previous LB episodes were reported by all participants. Details on tick exposure, the current LB manifestation and treatment were collected as well. Photographs of EM lesions were evaluated by two infectious diseases specialists and one dermatologist. New medical diagnoses were registered during follow-up. Baseline and follow-up questionnaires included questions on somatic symptoms (Patient Health Questionnaire, PHQ),[24] physical and social functioning (SF-36, subscales physical and social functioning),[25] health care use, absenteeism from work, illness perception (Brief-Illness Perception Questionnaire, B-IPQ),[26] cognitive and behavioral responses to symptoms (CBRSQ),[27] psychological distress (Hospital Anxiety and Depression Scale, HADS),[28] self-efficacy with respect to pain and fatigue (Self–Efficacy Scale, fatigue (SES-F), and Pain Catastrophizing Scale (PCS)),[29,30] and levels of physical activity (International Physical Activity Questionnaire, IPAQ).[31] At baseline and after six weeks, *B. burgdorferi* s.l.-specific antibodies were measured with a total Ig C6 ELISA (Immunetics, Boston, MA, USA), if borderline or positive followed by IgM and IgG immunoblot analyses (Mikrogen GmbH, Neuried, Germany). Serology for other tick-borne pathogens was performed using immunofluorescence analysis for detection of *Babesia microti*, *Rickettsia conorii*, and *Anaplasma phagocytophilum* antibodies (Focus Diagnostics, Cypress, CA, USA), IgM and IgG ELISA for the Tick borne encephalitis (TBE) virus (TestLine Clinical Diagnostics, Brno, Czech Republic). Positive TBE antibody results were confirmed by using an in-house virus neutralization assay. *B. miyamotoi* antibodies were assessed by an experimental protein array.[32,33] Real-time (multiplex) PCRs, based on various genes specific for *B. burgdorferi* s.l., *B. miyamotoi*, *A. phagocytophilum*, *Candidatus Neoehrlichia mikurensis*, spotted fever Rickettsia's, *Bartonella* spp., *Spiroplasma ixodetes*, TBEV, and a wide range of *Babesia* spp., were performed on all blood samples.[34] Skin biopsies were assessed for *B. burgdorferi* s.l. culture and PCR, and *B. burgdorferi* s.l. isolates from skin biopsies were typed by multilocus sequence typing (MLST). Whole blood samples and isolated peripheral blood mononuclear cells (PBMCs) were *ex vivo* stimulated with live attenuated *B. burgdorferi* s.l. (mix of *B. burgdorferi* sensu stricto ATCC strain 35210, *B. garinii* ATCC strain 51383, and *B. afzelii* pKo isolate) at different multiplicities of infection (MOI), lipopolysaccharide (purified LPS from *E. coli* serotype 055:B5; Sigma–Aldrich, St. Louis, MO, USA), Pam3Cys (EMC microcollections, Tübingen, Germany), and heat killed *Candida albicans* blastoconidia (ATCC MYA-3573, UC 820), and RPMI as a medium control. Concentrations of interleukin (IL)-1ß, IL-6, IL-1Ra and IL-10 in cell culture supernatants after 24 h of incubation were measured with commercial ELISA kits (Sanquin Reagents, Amsterdam, the Netherlands, and R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturers' instructions. In a subset of patients, three pre-market and commercial cellular tests were performed, including the Spirofind Revised (Oxford Immunotec, Oxford, UK), the Lyme iSpot (Autoimmun Diagnostika, Strassberg, Germany), and LTT-MELISA (InVitaLab, Neuss, Germany). The latter two are commercially available and were performed at facilities of their respective manufacturers in Germany.[21] DNA was isolated from EDTA whole blood samples for genome wide association studies (GWAS) using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's protocol. The presence of 13 predetermined single-nucleotide polymorphisms (SNPs, as described in Table S2) was assessed after genotyping using the Infinium Global Screening Array MD v1.0 (GSA) BeadChip (Illumina, San Diego, CA, USA). For the cohort of patients with chronic symptoms attributed to LB without confirmation, blood was collected at baseline and patients were subjected to the same measurements as the LB patients, with the exception of skin biopsies. Extensive questionnaire data from tick bite and population cohorts, similar to the LB cohort, were used for comparison.

Statistics

Statistical methods were pre-determined in a statistical analysis plan, and data handling was covered in a data management plan (Supplementary Appendix). Data were collected through a customized secured database at the online Tekenradar platform (www.tekenradar.nl). Sample size calculations were based on an assumed 5% prevalence of persistent symptoms. In a cohort of 1000 patients a 25% lower proportion for a determinant would be detected in patients without persistent symptoms if present in 77% of patients meeting the criteria for persistent symptoms, with a power of 80% and an alpha of 5%.[20] Confirmed LB patients were included in the analysis population if they had completed the symptom questionnaires (CIS, subscale fatigue, CFQ, and SF-36, subscale bodily pain) at least once. Missing data for the classification of having persistent symptoms or not were substituted by linear interpolation of preceding and following questionnaire scores. If first or final observations was missing, the first available questionnaire score was carried backward or the last available score was carried forward. Three alternative substitution methods were performed in sensitivity analyses (Table S3). The relationship of potential determinants ('explanatory variables') with persistent symptoms ('response variable') was studied both with multivariable analyses (random forest prediction analyses, RF), to assess collective predictive ability, and with association studies (AS). The RF algorithm was used to predict persistent symptoms with a combination of explanatory variables and possible confounders,[35–37] since this ensemble learning method performs very well when a large number of potential determinants are included. The direction and strength of the relation between explanatory and response variables were represented in partial dependence plots (PDP). The outcomes of the RF prediction algorithm were represented in a variable importance plot. Missing data for the analyses were substituted by neutral values, i.e. the lowest value in range minus 1. Variables that were available for only a minority of individuals were analysed in subgroup analyses. To complement the variable importance ranking, the relation of each explanatory variable with persistent symptoms was assessed in association studies. In these analyses, statistically significant differences were identified by permutation tests (Kruskal–Wallis tests for ordinal variables, and Cochran-Mantel-Haenszel for categorical variables). Potential confounders were taken into account. Correction for multiple testing was performed with the Benjamini-Hochberg method at a false discovery rate (FDR) of 10.0%,[38] and missing data for individual variables were not substituted. Finally, pairwise analyses of explanatory variables with the highest ranking or the most significant associations were performed to identify clusters of closely related variables contributing to persistence of symptoms. Non-parametric tests blocked by the potential confounders were used (Spearman for two continuous variables, Kruskal–Wallis for one ordinal or continuous and one categorical variable, Cochran-Mantel-Haenszel for two categorical variables, and sum statistics for an ordinal and an ordinal or continuous variable). The Benjamini-Hochberg method was used for multiple testing correction. Age, sex, educational level, and comorbidity were predefined as potential confounders for association studies and pairwise analyses, and values were categorized for stratification in blocks.[4] Three categories of educational levels were included in the current analyses: low (none or primary education), medium, and high (university of applied sciences and academic university education). In random forest analyses, these confounders were included as potential determinants. Moreover, four laboratory variables were included as potential determinants in RF and association studies to determine the influence of variance in the processing of blood samples (Table S2). For all analyses, R version 4.1.0 was used. Statistical analyses were performed using the *randomForest* and *coin* packages, and the *ggplot2* package was used for data visualization.

Ethics

Written informed consent was obtained from all participants. The study was performed in accordance with the Declaration of Helsinki, approved by the human subject review board Noord-Holland (NL50227.094.14).

Role of funders

The funding sources (ZonMw, Dutch Ministry of Health, Welfare and Sport) had no role in data collection, analysis and interpretation, report writing, or decision to submit for publication. All authors have full access to all data and take responsibility for submitting for publication.

Results

In total, 1135 patients with physician-confirmed LB were included in the analysis population, of whom 295 met the predefined criteria for persistent symptoms in one year of follow-up.[4] The majority of patients were included online through online questionnaires, enabling patients from all over the country to participate, and about 10% were included through clinical LB centers. Table 2 shows baseline patient characteristics. Data from 4000 individuals in the population cohort and 2405 individuals in the tick bite cohort were available for comparison (Figure S1). In addition, 65 patients were included in the cohort of patients with chronic symptoms attributed to LB without clinical or laboratory confirmation (Table S4).

Determinants of persistent symptoms in patients treated for physician-confirmed LB

Fig. 1 shows the variable importance plot of the 30 most predictive variables of persistent symptoms in LB

	LB patients not meeting the criteria for persistent symptoms (n = 789)[a]	LB patients meeting the criteria for persistent symptoms (n = 295)[a]	All LB patients in the analysis population (n = 1135)[a]
Sex—no. F/M (%)	438/351 (55.5/44.5)	200/95 (67.8/32.2)	670/465 (59.0/41.0)
Age (years)	56 [46–64]	54 [44–61]	55 [45–63]
LB manifestation—no. (%)			
EM	751 (95.2)	275 (93.2)	1076 (94.8)[b]
ACA	15 (1.9)	13 (4.4)	30 (2.6)
LNB	13 (1.6)	3 (1.0)	16 (1.4)
Lyme arthritis	9 (1.1)	3 (1.0)	12 (1.1)
Borrelial lymphocytoma	1 (0.1)	0 (0.0)	1 (0.09)
Early symptoms other than EM	0 (0.0)	1 (0.3)	1 (0.09)
LB duration (days)	6 [3–18]	6 [3–18]	6 [3–18]
Educational level—no. (%)			
Low	39 (4.9)	17 (5.8)	61 (5.4)
Medium	300 (38.0)	133 (45.1)	455 (40.1)
High	450 (57.0)	145 (49.2)	619 (54.5)
Number of comorbidities[c]	0 [0–1]	1 [0–2]	1 [0–1]
Baseline fatigue, cognitive impairment and pain scores			
Fatigue (CIS fatigue)	18 [11–30]	41 [34–48]	26 [14–28]
Cognitive impairment (CFQ)	24 [16–33]	37 [26–49]	27 [18–37]
Pain (SF-36 bodily pain)	89.8 [77.55–100]	67.35 [44.9–89.8]	89.8 [67.35–100]

Data are medians with interquartile ranges (IQR) for continuous values. Sex, age, educational level and number of comorbidities at baseline were predefined confounders in association studies. Differences in sex and number of comorbidities between both groups were significant, based on p-values as determined by chi-squared tests for categorical variables and student's t-test for continuous variables ($p < 0.001$). The significant difference for proportion of male/female was not relevant in the multivariable prediction analysis. CIS subscale fatigue scores ranged 8–56 (norm <35), a higher score indicating more fatigue. CFQ scores ranged 0–100 (norm <44), a higher score indicating more impaired cognitive functioning. SF-36 (subscale bodily pain) scores ranged 0–100 (norm >55), a higher score indicating less pain. Abbreviations: ACA = Acrodermatitis Chronic Atrophicans, EM = erythema migrans, LB = Lyme borreliosis, LNB = Lyme neuroborreliosis, no. = number, TIC-P = Treatment Inventory of Costs in Patients with psychiatric disorders. [a]The first two columns do not add up to n = 1135, since in the primary scenario for substitution of missing data, allocation of 51 patients was missing as they had completed less than two questionnaires. [b]The sum of manifestations exceeds the total number of patients in the analysis population, as one patient was diagnosed with two concurrent manifestations. [c]Self-reported comorbidities (using the TIC-P).

Table 2: **Baseline patient characteristics.**

patients as identified by random forest analysis. At the best overall performance, 71.0% of predictions in the primary analysis correctly indicated persistent symptoms, with a sensitivity of 73.0% and a specificity of 70.0%, and an area under the receiver operating characteristics (ROC) curve (AUC) of 0.79 (Figure S2). Of the 30 highest ranking variables, one was genetic (TLR10 polymorphism, ranked 30), and none were microbiological. Various immunological variables were predictive, although importance was at most moderate (ranked ≥12). Clinical characteristics of the current LB manifestation did not contribute substantially. Most important baseline predictors of persistent symptoms were impaired physical and social functioning (SF-36), higher depression and anxiety scores (HADS), higher brief-IPQ scores (reflecting more negative illness perceptions), and daily occupation (i.e., incapacity for work: Figure S3). Furthermore, number of baseline comorbidities, use of medication (categories of anti-inflammatory or anti-rheumatic drugs, and analgetics in particular), healthcare consumption, pre-existent anxiety and depression, and increase in number of somatic diagnoses other than LB during follow-up were general clinical predictors of persistent symptoms (Figure S4).

The relationship of each of the individual potential determinants with persistent symptoms was assessed in association studies. As with the RF results, baseline functional, psychosocial, and general clinical factors were particularly associated with persistent symptoms (FDR<0.10) (Table 3). Significant immunological variables were lower IL-10 production upon peripheral blood mononuclear cell (PBMC) stimulation with *B. burgdorferi* s.l. at six weeks (FDR<0.05), and a higher ratio between IL-10 production upon PBMC stimulation with lipopolysaccharide (LPS) relative to *B. burgdorferi* s.l. at six weeks (FDR 0.05–0.10). In line with the prediction results, the majority of microbiological, immunological and genetic variables, and clinical characteristics directly related to the LB manifestation, were not significantly associated with persistent symptoms. Some variables were not predictive in multivariable analyses, but were significantly associated individually, including less physical activity and longer antibiotic treatment for LB (FDR 0.05–0.10, RF: ranked 118 and 84, respectively). A higher number of missing

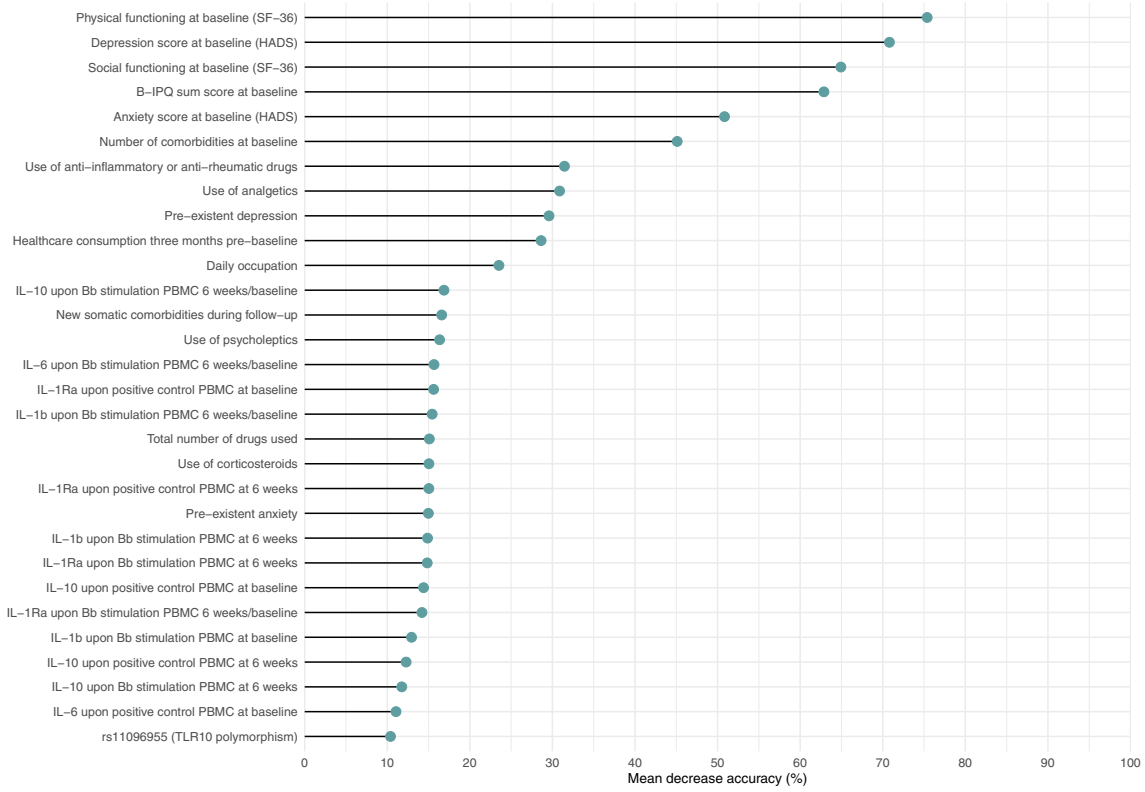

***Fig. 1:* Random forest variable importance plot**. Ranking of the 30 variables (out of a total of 159 variables) that were most important in the algorithm prediction process for persistent symptoms after treatment for LB in 1084 patients (who had completed the primary outcome questionnaires at ≥2 time points, and of whom 295 met the definition for persistent symptoms), as indicated by random forest analysis. For 71.0% of patients the presence of persistent symptoms was predicted correctly (sensitivity 73.0%, specificity 70.0%, ROC AUC 0.79). Mean decrease accuracy: how much the model accuracy decreases by excluding a given variable. Partial dependence plots are in Figure S4. Abbreviations: AUC = area under the curve, Bb = *Borrelia burgdorferi* s.l., B-IPQ = Brief-Illness Perception Questionnaire, HADS = Hospital Anxiety and Depression Scale, IL = interleukin, PBMC = peripheral blood mononuclear cell, ROC = receiver operating characteristic, SF-36 = SF-36 item Health Survey, TLR = Toll-like receptor.

observations (FDR<0.05, RF: ranked 62) suggested that patients with persistent symptoms completed fewer follow-up questionnaires than patients without persistent symptoms, although absolute numbers were small (Table S5). C6 IgM/IgG ELISA sero-reversion (a positive baseline result turning negative) was found only in 48 LB patients, and was significantly associated with not having persistent symptoms (FDR 0.05–0.10, RF: ranked 123).

Sensitivity and additional analyses in patients treated for physician-confirmed LB

Both results of sensitivity analyses using alternative scenarios for substitution of missing data, and sensitivity analyses in predefined subgroups of patients were comparable with those of the primary analyses (Table S3). In addition, when continuous severity scores for fatigue, cognitive impairment, or pain at six months were used as response variables, outcomes were largely similar to those based on persistent symptoms according to the definition. These sensitivity analyses confirmed robustness of the primary outcomes.

To rule out that the predictive value of healthcare visits in the three months pre-inclusion in the primary analysis was related to LB symptoms already existing before inclusion, a subgroup analysis in 525 patients with short duration of EM (≤5 day at treatment start) was performed. The number of healthcare visits in the past three months remained predictive of persistent symptoms.

Additional data were collected in the subgroup of patients who reported fatigue, cognitive impairment, or pain at baseline. These patients completed questionnaires on cognitive-behavioral responses to their symptoms (CBRSQ, PCS, and SES-F). Higher CBRSQ subscale scores (i.e., "all or nothing behavior", "fear avoidance", "avoidance/resting", and "catastrophizing"), higher pain catastrophizing (PCS) scores and lower self-efficacy with respect to fatigue (SES-F) predicted, and were associated with, persistent symptoms.

AS ranking	Variable	RF ranking	FDR level	FDR	p-value (95% CI)	Spearman's rank correlation coefficient
1	Pre-existent depression	9	<0.05	<0.0001	<0.0001	1
2	Physical functioning at baseline (SF-36)	1	<0.05	<0.0001	<0.0001	−1
3	Social functioning at baseline (SF-36)	3	<0.05	<0.0001	<0.0001	−1
4	B-IPQ sum score at baseline	4	<0.05	<0.0001	<0.0001	1
5	New somatic comorbidities during follow-up	13	<0.05	<0.0001	<0.0001	1
6	Anxiety score at baseline (HADS)	5	<0.05	<0.0001	<0.0001	0.263
7	Depression score at baseline (HADS)	2	<0.05	<0.0001	<0.0001	0.323
8	Healthcare consumption three months pre-baseline	10	<0.05	0.0002	<0.0001	1
9	Use of analgetics	8	<0.05	0.0008	<0.0001	1
10	Number of completed time points	62	<0.05	0.0012	<0.0001	−1
11	Use of anti-inflammatory or anti-rheumatic drugs	7	<0.05	0.0044	0.0003 (0.0003–0.0003)	1
12	Use of corticosteroids	19	<0.05	0.0118	0.0009 (0.0009–0.001)	0.237
13	IL-10 upon Bb stimulation PBMC at 6 weeks	28	<0.05	0.0215	0.0018 (0.0017–0.0019)	−0.086
14	Pre-existent anxiety	21	<0.05	0.0411	0.0037 (0.0036–0.0039)	0.095
15	Use of psycholeptics	16	0.05–<0.10	0.0507	0.0049 (0.0048–0.0051)	0.138
16	Daily occupation	11	<0.05	0.0483	0.005 (0.0049–0.0052)	NA[a]
17	Total number of drugs used	18	0.05–<0.10	0.0804	0.0087 (0.0087–0.0091)	0.075
18	IPAQ Metabolic minutes at baseline	118	0.05–<0.10	0.0796	0.0093 (0.0091–0.0095)	−0.074
19	ABX duration	84	0.05–<0.10	0.0872	0.0108 (0.0106–0.011)	0.056
20	IL-10 upon positive control PBMC at 6 weeks	27	0.05-<0.10	0.0921	0.012 (0.0117–0.0122)	0.073
21	Sero-reversion Bb C6 (IgM/IgG) ELISA	123	0.05–<0.10	0.0961	0.0131 (0.0129–0.0133)	0.124
35	IL-10 upon Bb stimulation PBMC 6 weeks/baseline	12	NS	0.3592	0.0816 (0.0811–0.0822)	-NA
42	IL-10 upon positive control PBMC at baseline	24	NS	0.4068	0.1109 (0.1103–0.1116)	NA
58	rs11096955 (TLR10 polymorphism)	30	NS	0.7751	0.292 (0.29–0.293)	-NA
69	IL-6 upon Bb stimulation PBMC 6 weeks/baseline	15	NS	0.8256	0.37 (0.369–0.3709)	NA
70	IL-1β upon Bb stimulation PBMC 6 weeks/baseline	17	NS	0.8262	0.3756 (0.3746–0.3765)	NA
83	IL-1β upon Bb stimulation PBMC at 6 weeks	22	NS	0.86	0.4635 (0.4625–0.4645)	NA
124	IL-1Ra upon Bb stimulation PBMC 6 weeks/baseline	25	NS	0.9687	0.78 (0.7792–0.7808)	NA
125	IL-6 upon positive control PBMC at baseline	29	NS	0.9725	0.7894 (0.7886–0.7902)	NA
127	IL-1Ra upon positive control PBMC at 6 weeks	20	NS	0.9791	0.8075 (0.8067–0.8082)	NA
128	IL-1Ra upon Bb stimulation PBMC at 6 weeks	23	NS	0.9765	0.8117 (0.8109–0.8124)	NA
136	IL-1β upon Bb stimulation PBMC at baseline	26	NS	0.9883	0.8728 (0.8721–0.8734)	NA
140	IL-1Ra upon positive control PBMC at baseline	16	NS	0.9918	0.9016 (0.901–0.9022)	NA
NA	Number of comorbidities at baseline	6	NA	NA	NA	NA

Variables significantly associated with persistent symptoms (variables 1 to 21), and p-values (for association studies of the variables that were in the 30 most important in the algorithm prediction process for persistent symptoms being not significant in association studies (remaining variables). AS ranking is based on the p-value (Kruskal–Wallis tests for ordinal variables, and Cochran-Mantel-Haenszel for categorical variables), and the Benjamini-Hochberg method for correction for multiple testing at the nominal false discovery rate (FDR) of 0.10 was applied. Spearman's rank correlation coefficient indicates a quantification and direction of the significant relationship between the explanatory variable and persistent symptoms. Number of baseline comorbidities was included as confounder in association studies, but not in RF. Abbreviations: ABX = antibiotic therapy, AS = association studies, Bb = *Borrelia burgdorferi*, B-IPQ = Brief-Illness Perception Questionnaire, CI = confidence interval, ELISA = Enzyme-Linked Immunosorbent Assay, FDR = false discovery rate, HADS = Hospital Anxiety and Depression Scale, IL = interleukin, IPAQ = International Physical Activity Questionnaire, NA = not applicable, NS = not significant, PBMC = peripheral blood mononuclear cell, RF = random forest, SF-36 = SF-36 item Health Survey, TLR = Toll-like receptor. [a]Spearman's rank correlation coefficient was not available because of >two categories; see Figure S3.

Table 3: **Variables significantly associated with persistent symptoms.**

When including baseline severity of fatigue, cognitive impairment, and pain as potential determinants, these were the strongest predictors of persistent symptoms after treatment for LB. When including all symptoms assessed at baseline as potential determinants, most were predictive, and associated with, persistent symptoms, including fatigue, concentration disorder, and arthralgia. Also, fever was significantly associated with persistent symptoms, although reported by only 69/451 patients, of whom 66 with EM.

The eight linear single-item B-IPQ subscale scores were included separately in an additional analysis, to further assess the meaning of illness perceptions in the primary analysis. Baseline scores for subscales Identity ("How much do you experience physical symptoms from LB"), Consequences ("How much does LB affect your life"), and Emotions ("How much does LB affect you emotionally") were mostly predicting and significantly associated with persistent symptoms after treatment for LB. When taken as the response variable, baseline B-IPQ

subscale Identity scores were predicted by lower baseline physical and social functioning and higher depression scores. Larger EM diameter, disseminated manifestations, and longer antibiotic treatment were predictors of B-IPQ Identity scores as well (Figure S5). For individuals in the tick bite cohort, who had not experienced LB and whose B-IPQ questions referred to their tick bite instead, subscales Emotions (ranked 15, FDR <0.05), Concern (ranked 17, NS), and Consequences (ranked 25, FDR <0.05) contributed to the prediction of persistent symptoms, but less prominently than in LB patients.

Determinants of persistent symptoms in reference cohorts in relation to patients treated for physician-confirmed LB

Predictors of persistent symptoms in the reference cohorts were largely similar to predictors in confirmed LB patients. Indeed, having a confirmed LB, as assessed in an analysis based on the LB and reference cohorts, was significantly associated with persistent symptoms, but only moderately predictive in multivariable prediction analysis (ranked 17).

Since the main determinants of persistent symptoms in patients treated for physician-confirmed LB in the primary analyses were rather generic and overlapping with determinants of persistent symptoms in reference cohorts, we performed additional analyses in LB patients trying to identify variables specifically related to *B. burgdorferi* s.l. infection. First, prediction analysis with only biometric variables (i.e., serological, genetic and immunological) did not predict persistent symptoms. Second, we assessed determinants of lower (first quartile) IL-10 production at six weeks in patients with persistent symptoms (n = 59), since lower IL-10 production upon *B. burgdorferi* s.l. stimulation of PBMCs at six weeks was predictive in the primary analysis. This resulted in a prediction model in which impaired social functioning and higher depression score were important, as well as polymorphisms for IL-1β, TLR10 and IL-10, and negative *B. burgdorferi* s.l. skin biopsies (PCR and culture). Third, we looked for determinants of attributing persistent symptoms during follow-up to LB. Of 295 patients with persistent symptoms, 188 (63.7%) did not attribute symptoms during follow-up to LB, potentially because of another explanation for these symptoms. By excluding patients with persistent symptoms due to other, known reasons, we hypothesized that findings would be more LB-specific. Baseline poorer physical and social functioning and higher B-IPQ scores were most predictive, as well as longer antibiotic treatment. Polymorphisms in MBL and two IL-1β promotor genes,[39,40] and disseminated LB were significantly associated with attribution of persistent symptoms to LB (Figure S6).

Pairwise associations in patients treated for physician-confirmed LB

Mutual dependencies were examined in patients treated for LB by testing the pairwise associations of the 15 variables with the highest ranking (RF) and the 15 most significantly associated variables, of which 11 were overlapping, and of the attribution of symptoms to LB (Figure S7). Poorer baseline social and (to a lesser extent) physical functioning were associated with higher anxiety scores, more negative illness perception (B-IPQ), and more healthcare consumption in the three months before inclusion. Attributing symptoms during follow-up to LB was highly associated with higher depression scores and poorer social functioning at baseline.

Determinants of persistent symptoms in patients with chronic symptoms attributed to LB without confirmation

Determinants identified in patients treated for physician-confirmed LB were assessed in a separate cohort of 65 patients who at baseline reported chronic (≥six months) symptoms of unknown origin that were attributed to LB, without clinical or laboratory confirmation. Criteria for persistent fatigue, cognitive impairment or pain in the one year after enrollment were met by 80.7% (46/57) of patients. Random forest analysis in this cohort lacked a clear ranking of importance (pmc 0.47, sensitivity 0.48, specificity 0.73), probably due to low numbers. The only significant association with persistent symptoms was absenteeism from paid work at baseline. Therefore, comparison with the primary analysis in patients with confirmed LB was deemed inappropriate.

Discussion

We assessed a wide range of factors potentially predictive of, or associated with, persistent symptoms after treatment for Lyme borreliosis. Both random forest analysis and association studies were performed in a prospective cohort of 1135 patients with confirmed LB, and in two reference cohorts of individuals without LB. Most important determinants were poorer social and physical functioning, more negative illness perceptions, and higher anxiety and depression scores at start of antibiotic treatment. *B. burgdorferi* s.l. infection-specific microbiological, immunological and genetic factors had at most limited predictive value for persistent symptoms. Clinical characteristics related to LB did not substantially contribute. More severe fatigue, cognitive impairment or pain at baseline were highly predictive. In patients with symptoms at baseline, cognitive-behavioral responses to symptoms contributed to the prediction of symptom persistence as well.

Previously, we reported a prevalence of persistent fatigue, cognitive impairment, or pain of 27.2% in this same LB cohort, being 3.9% and 6.0% higher than in two reference cohorts of individuals without LB.[4] Thus, although symptoms were significantly more prevalent after treatment for LB, in the majority of LB patients these symptoms are likely explained by background

prevalence rather than by *B. burgdorferi* s.l. infection. In the current study, this was supported by an additional analysis combining LB and reference cohorts: being diagnosed with LB was only a moderate predictor of persistent symptoms, whereas main determinants were generic.

Of the five most important predictors of persistent symptoms in LB patients, the Brief Illness Perception Questionnaire (B-IPQ) score was the only variable specifically focusing on LB. This questionnaire covers daily and emotional consequences, expected timelines, personal control, expected treatment effect, symptoms, concerns, and understanding of illness (i.e., LB). More negative scores predicted persistent symptoms after LB. Additional analysis revealed that the Identity subscale ("How much do you experience physical symptoms from LB") was most important. To interpret its predictive value, we zoomed in on the Identity subscale and found that predictors of this scale itself included larger EM size and disseminated LB. Both of these reflect more obvious symptoms due to LB, but were not predictive of persistent symptoms in the primary analyses. In the tick bite cohort, i.e., in individuals without LB, this subscale was not predictive of persistent symptoms. Altogether, to what extent the predictive value of B-IPQ in the primary analysis reflected more negative illness perceptions, perhaps being more pronounced in patients with more obvious LB symptoms, or the actual burden of the LB manifestation, remained indecisive.

Mechanisms underlying development of persistent symptoms may have been heterogeneous, including infection-specific and transcending factors. This may be one of the reasons why trans-diagnostic, more generic factors, such as depression, anxiety, comorbidity, and beliefs and behavior in response to symptoms, have been associated with long-lasting fatigue in previous studies as well.[41] The association of depression and anxiety scores with persistent symptoms after LB does not prove a direct relation, but rather indicates that psychological distress from any origin may be accompanied by long-lasting symptoms. Nevertheless, associations between baseline anxiety or depression and long-term outcomes have been suggested for various illnesses, including COVID-19, although they have not been confirmed in all studies.[42,43] Beliefs and behavior, as reflected by CBRSQ subscales, have been described to mediate change by behavioral interventions in several long-term medical conditions,[44] and our findings may give rise to careful evaluation of potential beneficial effects of interventions focusing on cognitive-behavioral responses to symptoms in some patients with persistent symptoms after treatment for LB. Similar to the LB cohort, impaired physical and social functioning, and comorbidity were determinants of persistent symptoms in the reference cohorts, indicating an infection-independent mechanism. Altogether, the finding of trans-diagnostic factors as main determinants of persistent symptoms after LB corresponds with literature on chronic illnesses, and may relate to the considerable prevalence of these symptoms in general, or indicate shared underlying mechanisms.

We hypothesized that there possibly is a direct relation of persistent symptoms with the *B. burgdorferi* s.l. infection in some patients. Because of potential therapeutic, preventive or prognostic consequences, we performed additional analyses attempting to identify these patients and LB-specific determinants of persistent symptoms. We analysed a subgroup of patients who self-attributed symptoms during follow-up to LB, and found association with a MBL polymorphism, a C-type lectin involved in complement activation after pathogen recognition. MBL2-deficiency in mice has been associated with a more severe course of various infectious diseases, and with a higher number of *B. burgdorferi* spirochetes in the skin and higher IgG antibody responses.[40] Considering that longer antibiotic treatment and disseminated LB also associated with self-attributing long-term symptoms to LB, persistent symptoms could have related with more severe *B. burgdorferi* s.l. infection in this patient subgroup. Additionally, we zoomed in on lower IL-10 responses, since we found that lower IL-10 production in stimulation experiments at six weeks predicted, and associated with, persistent symptoms. IL-10, an anti-inflammatory cytokine, plays an important role in balancing pro-inflammatory and anti-inflammatory immune responses. In acute *B. burgdorferi* s.l infection, lower IL-10 responses may relate to higher pro-inflammatory responses and increased *B. burgdorferi* s.l. clearance.[45] This aligns with the associations we found with negative baseline skin biopsies and polymorphisms in TLR1 and TLR10 that have been associated with higher pro-inflammatory responses in LB.[13,46] Whether IL-10 responses actually play a role in mechanisms involved in persistent symptoms after treatment for LB remains to be elucidated in future studies.

Part of the determinants reported here have previously been described in LB, including anxiety[47] and illness perceptions (particularly B-IPQ subscales Identity and Consequences).[48] In accordance with previous studies, EM size,[47] disseminated LB,[49] number of symptoms at initiation of antibiotic therapy,[49] and seropositivity[49] were not contributing. Likewise, as in another study,[50] we found no association of persistent symptoms with other tick-borne diseases, which could however be due to small numbers of test positives. For that reason, the association of baseline fever with persistent symptoms, may particularly have related to LB itself. We found no predictive value of LB symptom duration before antibiotic treatment, with a short median duration of five days (range 0–2978 days), due to a majority of EM patients. Whereas duration of symptoms > six weeks was associated with persistent symptoms in a prospective study in Lyme

neuroborreliosis,[51] other studies found no association as well.[47,49] Specific SNPs that have previously been associated with persistent symptoms were not confirmed in the current study (Table S2). Unlike our observation, prevalence of sero-reversion was not significantly different between PTLDS and non-PTLDS patients in a previous study.[47] Differences may relate to study design (prospective versus retrospective), statistical methods (univariable versus multivariable), numbers of potential determinants assessed, definition of determinants, population differences (e.g., Europe versus North America), cohort sizes, and case definitions.

Our study has several limitations. First, results were based on intrinsically self-reported symptoms and thus subjective rather than objective. However, assignment to persistence was based on scoring in the impaired range of standardised and validated questionnaire systems that are considered best-of-class in this research area. Sensitivity analyses on various scenarios for substitution of missing data revealed similar results. Second, we pre-defined potential determinants based on literature. Results are restricted to included factors, whereas a role for other factors cannot be excluded. Based on recent literature, measurement of serum cytokines or chemokines,[49,52,53] and metabolomic pathways[54] could have had additional value. Third, the relationship between determinants and persistent symptoms, i.e., cause and effect, and the interpretation remains uncertain. Our study design and the high prevalence of such symptoms in the reference cohorts prevents us from drawing firm conclusions regarding this. Additionally, based on the current explorative study, the absence of an association does not imply that the association does not exist. We interpreted the findings in light of existing background knowledge and consider these to be hypothesis-generating. Fourth, because the number of patients with disseminated LB was relatively small, determinants described particularly relate to patients with EM. Last, our criteria for persistent symptoms did not include functional disability, in contrast to the formal PTLDS criteria, although, we observed that physical and social functioning was impaired in substantial part of those patients.[4]

Enrollment in this study was possible through online self-registration after physician-confirmed diagnosis of LB, and through the participating clinical LB centers for all patients with a physician-confirmed LB diagnosis. One could hypothesize that online self-registration of patients may be prone to selection bias. However, primary outcome in terms of the prevalence of persistent symptoms after treatment for LB was comparable for patients who had self-registered and patients who were consecutively included by physicians. Strength of the study was augmented by the comparison with reference cohorts, one of which was recruited similarly through the online study platform. Furthermore, robustness of results was confirmed by sensitivity analyses. And finally, the cohort of patients treated for physician-confirmed LB reflected the nationwide incidence of LB, in terms of the distribution of age, sex, and physician-reported LB manifestations (3–6% disseminated LB).[55,56]

In summary, determinants of persistent symptoms after LB were mostly generic, including baseline functioning, comorbidity, and baseline symptom severity. This might well be explained by the substantial prevalence of these symptoms in reference cohorts, indicating that persistent symptoms could not be attributed to *B. burgdorferi* s.l. infection in a large part of the LB cohort. We did observe that cognitive-behavioral factors may have a role in persistence of symptoms, and reported immunological and genetic factors that predict, and might contribute to the development of, persistent symptoms in a subset of patients. As many patients with persistent symptoms experience disabling symptoms and at current we can only offer supportive care, our findings might provide direction to further research on potential underlying mechanisms of persistent symptoms after treatment for LB, particularly immunological responses.

Contributors

HDV, JU, and CCvdW were primarily responsible for data collection, curation, analyses and writing of the original draft of the manuscript. MGH, ADT, MEB, FRvdS, SAG, TPZ, and YMV contributed to patient inclusion and data collection. JAF contributed to methodology, data analysis and interpretation. HS and KK arranged molecular and serological laboratory measurements, respectively. HK, LABJ, BJK, JWH and CCvdW conceived, designed, and supervised the study project. HDV, JU, and CCvdW verified underlying data. All authors read and approved the final version of the manuscript.

Data sharing statement

The study protocol and statistical analysis plan are provided in the supplemental data. De-identified data that underlie the results of the current study can be obtained upon request for a period of 36 months after publications of intended manuscripts. Qualified researchers with appropriate ethics board approvals and data use agreements can request for data and work flow programming documents by contacting the corresponding author by e-mail.

Declaration of interests

MEB, FRvdS and JWH report collaborations on LB diagnostics with Pfizer, Antigen Discovery Inc., Oxford Immunotec, AID/GenID, and InVitaLab; funding to institution and/or material supplied for research free of charge, no personal financial compensation. MEB reports support for travel expenses related to LB symposium at Massachusetts General Hospital. LABJ and BJK are co-inventor of Spirofind, an experimental in-house assay for Lyme borreliosis, which is owned by Radboudumc and will not be developed further as potential diagnostic test. CCvdW is a consortium partner and partner in sub-project in the NMCB consortium (The Dutch ME/CFS Cohort and Biobank Consortium) funded by Netherlands Organization for Health Research and Development (ZonMw, project numbers 10091012110027 and 10091012110018). The other authors report no competing interests.

Acknowledgements

We gratefully acknowledge the following persons for their contributions to this study: G.H.J. van Arkel, M. Becker-Stynenbosch, R. van Boxtel, M.A.E. Brouwer, H. Dijkstra, A.R.T. Donders, J. Ersöz, E. Franz, I. Friesema, D. de Haan, R. Hartman, L. van der Hoeven, H. ter Hofstede,

M.N. Hoogerwerf, H. ten Hulscher, M. Jonker, M. Kerstholt, B. Koetsier, B. van Kooten, B.A. Lemkes, H. Lemmers, C.D.M. Nijhuis, G.A. Oei, J.H.J. Reimerink, M. Starink, C. Uittenbosch, *A. van* Vliet, H. van Weert, A. Wijmenga, M. Zwerink; and all the study participants. The LymeProspect study was funded by the Netherlands Organisation for Health Research and Development, (ZonMw) (project number 522001003), and the Dutch Ministry of Health, Welfare and Sport, (VWS).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104825.

References

1 Wormser G, Dattwyler R, Shapiro E, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis*. 2006;43(9):1089–1134.
2 Aucott JN, Crowder LA, Kortte KB. Development of a foundation for a case definition of post-treatment Lyme disease syndrome. *Int J Infect Dis*. 2013;17(6):e443–e449.
3 Rebman AW, Bechtold KT, Yang T, et al. The clinical, symptom, and quality-of-life characterization of a well-defined group of patients with posttreatment Lyme disease syndrome. *Front Med (Lausanne)*. 2017;4:224.
4 Ursinus J, Vrijmoeth HD, Harms MG, et al. Prevalence of persistent symptoms after treatment for Lyme borreliosis: a prospective observational cohort study. *Lancet Reg Health Eur*. 2021;6:100142.
5 Borsic K, Blagus R, Cerar T, Strle F, Stupica D. Clinical course, serologic response, and long-term outcome in elderly patients with early Lyme borreliosis. *J Clin Med*. 2018;7(12):506.
6 Wormser GP, Shapiro ED. Implications of gender in chronic Lyme disease. *J Womens Health (Larchmt)*. 2009;18(6):831–834.
7 Weitzner E, McKenna D, Nowakowski J, et al. Long-term assessment of post-treatment symptoms in patients with culture-confirmed early Lyme disease. *Clin Infect Dis*. 2015;61(12):1800–1806.
8 Ljostad U, Mygland A. Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. *Eur J Neurol*. 2010;17(1):118–123.
9 Stupica D, Lusa L, Maraspin V, et al. Correlation of culture positivity, PCR positivity, and burden of Borrelia burgdorferi sensu lato in skin samples of erythema migrans patients with clinical findings. *PLoS One*. 2015;10(9):e0136600.
10 Sjowall J, Fryland L, Nordberg M, et al. Decreased Th1-type inflammatory cytokine expression in the skin is associated with persisting symptoms after treatment of erythema migrans. *PLoS One*. 2011;6(3):e18220.
11 Embers ME, Barthold SW, Borda JT, et al. Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. *PLoS One*. 2012;7(1):e29914.
12 Krause PJ, Telford SR 3rd, Spielman A, et al. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. *JAMA*. 1996;275(21):1657–1660.
13 Oosting M, Ter Hofstede H, Sturm P, et al. TLR1/TLR2 heterodimers play an important role in the recognition of Borrelia spirochetes. *PLoS One*. 2011;6(10):e25998.
14 Hein TM, Sander P, Giryes A, Reinhardt JO, Hoegel J, Schneider EM. Cytokine expression patterns and single nucleotide polymorphisms (SNPs) in patients with chronic borreliosis. *Antibiotics (Basel)*. 2019;8(3):107.
15 Hassett AL, Radvanski DC, Buyske S, Savage SV, Sigal LH. Psychiatric comorbidity and other psychological factors in patients with "chronic Lyme disease". *Am J Med*. 2009;122(9):843–850.
16 Vercoulen JH, Swanink CM, Galama JM, et al. The persistence of fatigue in chronic fatigue syndrome and multiple sclerosis: development of a model. *J Psychosom Res*. 1998;45(6):507–517.
17 Morroy G, Keijmel SP, Delsing CE, et al. Fatigue following acute Q-fever: a systematic literature review. *PLoS One*. 2016;11(5):e0155884.
18 Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ*. 2006;333(7568):575.
19 Ballering AV, van Zon SKR, Olde Hartman TC, Rosmalen JGM, Lifelines Corona Research I. Persistence of somatic symptoms after COVID-19 in The Netherlands: an observational cohort study. *Lancet*. 2022;400(10350):452–461.
20 Vrijmoeth HD, Ursinus J, Harms MG, et al. Prevalence and determinants of persistent symptoms after treatment for Lyme borreliosis: study protocol for an observational, prospective cohort study (LymeProspect). *BMC Infect Dis*. 2019;19(1):324.
21 Baarsma ME, van de Schoor FR, Gauw SA, et al. Diagnostic parameters of cellular tests for Lyme borreliosis in Europe (VICTORY study): a case-control study. *Lancet Infect Dis*. 2022;22(9):1388–1396.
22 Bouwmans C, De Jong K, Timman R, et al. Feasibility, reliability and validity of a questionnaire on healthcare consumption and productivity loss in patients with a psychiatric disorder (TiC-P). *BMC Health Serv Res*. 2013;13:217.
23 Heins MJ, Knoop H, Lobbestael J, Bleijenberg G. Childhood maltreatment and the response to cognitive behavior therapy for chronic fatigue syndrome. *J Psychosom Res*. 2011;71(6):404–410.
24 Stegenga BT, Kamphuis MH, King M, Nazareth I, Geerlings MI. The natural course and outcome of major depressive disorder in primary care: the PREDICT-NL study. *Soc Psychiatry Psychiatr Epidemiol*. 2012;47(1):87–95.
25 Stewart A, Hays R, Ware JJ. The MOS short-form general health survey. Reliability and validity in a patient population. *Med Care*. 1988;26(7):724–735.
26 Broadbent E, Petrie KJ, Main J, Weinman J. The brief illness perception questionnaire. *J Psychosom Res*. 2006;60(6):631–637.
27 Skerrett TN, Moss-Morris R. Fatigue and social impairment in multiple sclerosis: the role of patients' cognitive and behavioral responses to their symptoms. *J Psychosom Res*. 2006;61(5):587–593.
28 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand*. 1983;67(6):361–370.
29 Sullivan MJL, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. *Psychol Assess*. 1995;7(4):524–532.
30 Nicholas MK. *Self-efficacy and chronic pain*. St Andrews: Annual Conference of the British Psychological Society; 1989.
31 Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381–1395.
32 Koetsveld J, Kolyasnikova NM, Wagemakers A, et al. Serodiagnosis of Borrelia miyamotoi disease by measuring antibodies against GlpQ and variable major proteins. *Clin Microbiol Infect*. 2018;24(12):1338.e1–1338.e7.
33 Hoornstra D, Stukolova OA, Karan LS, et al. Development and validation of a protein array for detection of antibodies against the tick-borne pathogen Borrelia miyamotoi. *Microbiol Spectr*. 2022;10(6):e0203622.
34 Geebelen L, Lernout T, Tersago K, et al. No molecular detection of tick-borne pathogens in the blood of patients with erythema migrans in Belgium. *Parasit Vectors*. 2022;15(1):27.
35 Breiman. Random forests. *Mach Learn*. 2001;45(1):5–32.
36 Liaw A, Wiener M. Classification and regression by random forest. *R News*. 2002;2(3):18–22.
37 Biau G, Scornet E. A random forest guided tour. *Test*. 2016;25(2):197–227.
38 Ferreira J, Berkhof J, Souverein O, Zwinderman K. A multiple testing approach to high-dimensional association studies with an application to the detection of associations between risk factors of heart disease and genetic polymorphisms. *Stat Appl Genet Mol Biol*. 2009;8(1):1–56.
39 Khazim K, Azulay EE, Kristal B, Cohen I. Interleukin 1 gene polymorphism and susceptibility to disease. *Immunol Rev*. 2018;281(1):40–56.
40 Coumou J, Wagemakers A, Narasimhan S, et al. The role of Mannose Binding Lectin in the immune response against Borrelia burgdorferi sensu lato. *Sci Rep*. 2019;9(1):1431.
41 Goertz YMJ, Braamse AMJ, Spruit MA, et al. Fatigue in patients with chronic disease: results from the population-based Lifelines Cohort Study. *Sci Rep*. 2021;11(1):20977.
42 Bottemanne H, Gouraud C, Hulot JS, et al. Do anxiety and depression predict persistent physical symptoms after a severe COVID-19 episode? A prospective study. *Front Psychiatry*. 2021;12:757685.
43 Verveen A, Wynberg E, van Willigen HDG, et al. Severe fatigue in the first year following SARS-CoV-2 infection: a prospective cohort study. *Open Forum Infect Dis*. 2022;9(5):ofac127.
44 de Gier M, Picariello F, Slot M, et al. The relation between cognitive-behavioural responses to symptoms in patients with long

term medical conditions and the outcome of cognitive behavioural therapy for fatigue - a secondary analysis of four RCTs. *Behav Res Ther*. 2023;161:104243.
45 Lazarus JJ, Meadows MJ, Lintner RE, Wooten RM. IL-10 deficiency promotes increased Borrelia burgdorferi clearance predominantly through enhanced innate immune responses. *J Immunol*. 2006;177(10):7076–7085.
46 Oosting M, Cheng SC, Bolscher JM, et al. Human TLR10 is an anti-inflammatory pattern-recognition receptor. *Proc Natl Acad Sci U S A*. 2014;111(42):E4478–E4484.
47 Wormser GP, McKenna D, Shaffer KD, Silverman JH, Scavarda C, Visintainer P. Evaluation of selected variables to determine if any had predictive value for, or correlated with, residual symptoms at approximately 12 months after diagnosis and treatment of early Lyme disease. *Diagn Microbiol Infect Dis*. 2021;100(3):115348.
48 Hill EM, Frost A. Illness perceptions, coping, and health-related quality of life among individuals experiencing chronic Lyme disease. *Chronic Illn*. 2020;18(2):426–438.
49 Aucott JN, Soloski MJ, Rebman AW, et al. CCL19 as a chemokine risk factor for posttreatment lyme disease syndrome: a prospective clinical cohort study. *Clin Vaccine Immunol*. 2016;23(9):757–766.
50 Eliassen KE, Ocias LF, Krogfelt KA, et al. Tick-transmitted co-infections among erythema migrans patients in a general practice setting in Norway: a clinical and laboratory follow-up study. *BMC Infect Dis*. 2021;21(1):1044.
51 Eikeland R, Mygland A, Herlofson K, Ljostad U. Risk factors for a non-favorable outcome after treated European neuroborreliosis. *Acta Neurol Scand*. 2013;127(3):154–160.
52 Strle K, Stupica D, Drouin EE, Steere AC, Strle F. Elevated levels of IL-23 in a subset of patients with post-Lyme disease symptoms following erythema migrans. *Clin Infect Dis*. 2014;58(3):372–380.
53 Hernandez SA, Ogrinc K, Korva M, et al. Association of persistent symptoms after Lyme neuroborreliosis and increased levels of interferon-alpha in blood. *Emerg Infect Dis*. 2023;29(6):1091–1101.
54 Fitzgerald BL, Graham B, Delorey MJ, et al. Metabolic response in patients with post-treatment Lyme disease symptoms/syndrome. *Clin Infect Dis*. 2020;73(7):e2342–e2349.
55 Hofhuis A, Harms M, Bennema S, van den Wijngaard CC, van Pelt W. Physician reported incidence of early and late Lyme borreliosis. *Parasit Vectors*. 2015;8:161.
56 Houben E, de Jong H, Penning-van Beest F, et al. Incidence of Lyme borreliosis in the Dutch general practice population: a large-scale population-based cohort study across the Netherlands between 2015 and 2019. *Vector Borne Zoonotic Dis*. 2023;23(4):230–236.