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Protocol for a longitudinal cohort study of Lyme disease with physical, mental and immunological assessment

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ABSTRACT

Introduction There are limited data on the longitudinal impact of Lyme disease. Predictors of recovery have not been fully established using validated data collection instruments. There are sparse data on the immunological response to infection over time.

Methods and analysis This study is a longitudinal cohort study that will recruit 120 participants with Lyme disease in Ontario and Nova Scotia, Canada, with follow-up for up to 24 months. Data will be collected using the Short-Form 36 physical and mental component summaries, Depression and Anxiety Severity Scale Questionnaire, Fatigue Severity Scale and a battery of neuropsychological tests. Mononuclear cells, gene expression and cytokine profiling from blood samples will be used to assess immunological response. Analyses will include the use of non-linear mixed-effects modelling and proportional hazards models.

Ethics and dissemination Ethics approval has been obtained from ethics boards at McMaster University (Hamilton Integrated Research Ethics Board) (7564), Queens University (EMD 315-20) and Nova Scotia Health Research Ethics Board (1027173), and the study is enrolling participants. Written informed consent is obtained from all participants. The results will be disseminated by publication in a peer-reviewed journal and presented at a relevant conference. A brief report will be provided to decision-makers and patient groups.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study will comprehensively address long-term physical and mental functioning in Lyme disease.
⇒ The study will provide a global assessment of immunological response to Lyme disease.
⇒ Some potential participants might not want to participate because of the comprehensive nature of the testing.

INTRODUCTION

The incidence of Lyme disease (LD), caused by the bacteria *Borrelia burgdorferi* and transmitted by the bite of black legged Ixodes ticks, has been increasing in Canada.[1–3] Early localised infection typically results in a rash known as erythema migrans (EM), and, if left untreated, can progress to disseminated illness which can result in multiple skin lesions as well as neurological, cardiac (heart block), or joint involvement.[4–8]

Despite the conduct of cohort studies in the USA and elsewhere,[9–15] there is still limited information on the longitudinal impact of LD. Questions about the immunological response to infection over time remain largely unanswered. In particular, correlations between such a response and immune profiles have not been assessed. Predictors of recovery have not been fully established using validated data collection instruments, and the role of coinfection in long-term prognosis is uncertain. A longitudinal cohort study will not only address these questions but will also serve as a platform to address questions about diagnostic testing, long-term economic burden, and risk reduction.

METHODS AND ANALYSIS

Study design and objectives

This is a prospective cohort study.

The primary objective of this cohort study is to describe patterns of physical and mental outcomes of LD, and to assess predictors of long-term outcomes and factors associated with delayed recovery. Patient-relevant outcomes include physical and mental functioning, fatigue, depression and neurocognition.

Hypotheses

1. Among participants with early localised LD, early disseminated LD and late disseminated LD, early antibiotic treatment will lead to a faster recovery and reduce the risk of sequelae.
2. Pre-existing comorbidity and severity of illness at presentation are associated with delayed recovery.
3. Serum cytokine profiles indicative of inflammatory response (eg, Interleukin 1

beta (ILB), IL-12, IL-18, tumour necrosis factor (TNF), interferon (IFN)-γ) and excessive T-helper 17 cell response (ie, immunological response) are associated with delayed recovery while cytokines associated with anti-inflammatory response (IL-6, IL-10) are associated with shorter recovery.

4. As a hypothesis-generating component of this cohort study, we will assess whether transcriptome analysis of total RNA sequencing (obtained from blood) will lead to RNA profiles that are associated with LD prognosis.
5. Diagnosis of LD incurs short and long-term psychosocial and economic cost to patients, the healthcare system and society.

In order address the study questions, we are conducting a longitudinal cohort study. A primary LD cohort, defined by participants that meet criteria for LD (as defined below) will be followed. A healthy cohort will also be followed as a comparator. These cohorts are described below.

Study setting

The study is being conducted at emergency departments and clinics affiliated with Queen's University in Kingston, Ontario, and in Lunenburg, Nova Scotia, Canada.

Eligibility criteria

LD cohort

Adults (≥18 years) with:

Early localised LD which is defined by having both of the following:

i. History of exposure to endemic area
ii. Physician confirmed EM of >5 cm (which is photographed) where assessment includes a history of centrifugal expansion

Early disseminated LD which is defined by having all three of the following:

i. History of exposure to endemic area
ii. <3 months since symptom onset
iii. Positive serology (using CDC criteria) OR >1 EM lesion*

AND

iv. At least one of the following:

Carditis manifested by heart block OR neurological abnormalities (seventh cranial nerve palsy) OR radiculitis OR lymphocytic meningitis

AND

v. No alternate diagnosis

*If MD diagnosed does not require serology

Late disseminated LD which is defined by having all three of the following:

i. History of exposure to endemic area
ii. >3 months since symptom onset
iii. Positive serology (using CDC criteria)

AND

iv. At least one of the following:

asymmetric oligoarticular arthritis OR polyneuropathy

AND

v. No alternate diagnosis

We will exclude the following participants:

i. Immunocompromised (either due to a medical condition or due to immunosuppressive medications).
ii. Treated for an illness unrelated to LD that could, in the treating physician's opinion, interfere with interpretation of the outcome measures (eg, use of antibiotics for an indication other than LD).
iii. Previous diagnosis of LD confirmed by MD diagnosis of EM or compatible clinical symptoms and positive serology using CDC.
iv. Unable to participate in long-term follow-up.
v. Unable to answer questions because of linguistic or cognitive difficulty
vi. Travel history to an endemic region for LD outside of Canada and the USA within 30 days prior to date of enrollment.

Healthy control cohort

We will recruit a cohort of healthy controls who will be followed at the same time points as the other cohorts. This group will serve as a comparison group for diagnostic testing, immunological testing, and LD risk factor assessment. We anticipate enrolling at least 30 such controls through advertising in local newspapers and through primary care offices.

Adults (≥18 years) who meet all of the following:

i. Not being treated for an illness that could interfere with interpretation of the outcome measures.
ii. Able to participate in long-term follow-up.
iii. Able to answer questions (ie, have no linguistic or cognitive difficulty)
iv. No travel history to an endemic region for LD outside of Canada and the United States within 30 days prior to date of enrolment.

We will maintain a log of all patients approached about the study (including basic demographic data, eg, age, sex) to assess differences between enrolled and non-enrolled eligible patients for both cohorts.

Laboratory, emergency department, outpatient and medical records will be used to confirm the case definition. Demographic information, medical history, clinical signs and symptoms (including details of tick exposure), physical examination and current medications will be obtained from all participants.

Education and income will be obtained as an assessment of socioeconomic status. We will also collect out-of-pocket costs from participants and information on work loss using a costing questionnaire. Clinical care, including antibiotic use and other prescriptions will be at the discretion of the attending physicians but will be recorded. Clinical laboratory tests, electrocardiograms, spinal fluid analysis, joint fluid analysis and radiological assessments (MRI or CT) will be done at the discretion of the attending physician as part of routine clinical care. Although these procedures are not part of the study, the results will be recorded if performed. In patients with EM, prior to initiation of antibiotics, a punch biopsy, which is not part of routine care, may be requested for PCR for a

diagnostics study with separate informed consent. Specimens for serology (IgM, IgG) will be obtained as part of routine care. Spinal fluid and joint fluid will be obtained as part of routine care for diagnostics testing and will be saved for cytokine testing (ie, CXCL13). Participants in the cohort study will need to provide consent for these specimens to be tested for PCR for *Borrelia*. Peripheral blood mononuclear cells (PBMCs) will be obtained for immune phenotyping and RNA profiling.

Outcomes

A trained research associate or research nurse will assess physical and mental functioning outcomes on enrolment into the study (baseline visit) and, with respect to the baseline visit, on day 30, 3, 6, 9, 12 and 24 months. Assessments will be made in ambulatory care settings at both study sites. We will capture LD major sequelae of carditis and arthritis as well as length of any hospital stay and death.

Physical functioning

Physical functioning will be measured using the Physical Component Summary (PCS) of the Short-Form 36 (SF-36).[16 17] The SF-36 measures eight health constructs using eight scales with 2–10 items per scale (total of 36 questions). For the PCS, very high scores indicate no physical limitations, disabilities or decrements in well-being as well as high energy level. Very low scores indicate substantial limitations in self-care, physical, social and role activities; severe bodily pain or frequent tiredness. Although the raw scores range from 0 to 100 for the SF-36, these scores are adjusted for population norms using a linear transformation such that the mean for each subscale and summary scale is 50 with a SD of 10. The PCS scores are standardised to the general US population, allowing clear normative interpretation.

Mental functioning

To assess mental functioning, we will use the Mental Component Summary (MCS) of the SF-36, where very high scores indicate frequent positive affect, absence of psychological distress and of limitations in usual social/role activities due to emotional problems. Very low scores indicate frequent psychological distress, and substantial social and role disability due to emotional problems. The MCS scores are also standardised to the general US population (mean score, 50; SD, 10).

Depression and fatigue

We will use the Depression and Anxiety Severity Scale (DASS) and the Fatigue Severity Scale (FSS) to capture depressive symptoms and persistent fatigue. The DASS is a 14-item scale that includes an assessment of dysphoria, lack of interest or involvement. Scores are summed with a possible range from 0 (no symptoms) to 42. When administered to a general adult population, 80% of people have a score ≤9 or less and 70% a score ≤6. The FSS measures the perceived level of fatigue using a Likert scale where the score ranges from 1 (low fatigue level) to 7 (high fatigue level). Two-thirds of the general population will have a score between 2.7 and 5.3.

Cognitive functioning

A brief, yet comprehensive set of neuropsychological tests will be administered to each participant. These will be administered at the following points: baseline, 3, 6, 12 and 24 months. The following domains of cognitive functioning will be assessed: verbal learning and memory, visual learning and memory, executive functioning, attention and concentration, and speed of information processing. A symptom validity measure is included as a means of gauging adequate 'cognitive effort' or 'engagement' during the assessment. Tests were selected to represent the domains of cognitive functioning to be studied and were consistent with the consensus of published test compendia. Given the nature of the study, tests were also selected for brevity, portability, ease of administration and suitability for repeat administrations. The following tests will be used:

The Hopkins Verbal Learning Test-Revised (HVLT-R) is a 12-item list presented orally for three learning trials and immediately following each presentation, the participant is asked to recall as many words from the list as possible.

The Brief Visuospatial Memory Test-Revised (BVMT-R) is a measure of visual–spatial learning and memory for a matrix of six abstract figures. The figures are held before the participant for 10 s and then the participant is asked to reproduce the designs using paper and pencil.

The Tower of London-DX 2nd Edition assesses executive function. The examiner uses one tower and a set of beads to display the desired goal and the participant rearranges a second set of beads on a second tower to match the examiner's configuration.

The Stroop Color and Word Test (SCWT) is a measure of selective attention and response inhibition. The participant is required to inhibit competing information while making automatic reading responses to maintain attention on the target stimuli.

The Symbol Digits Modalities Test (SDMT) is a speed of information processing task requiring complex scanning and visual tracking. A series of nine symbols are paired with a single digit in a key at the top of a sheet of paper, with the remainder of the page presenting a randomised sequence of symbols. The participant is required to respond by voicing the digit associated with each symbol as quickly as possible within a 90 s time limit.

The Test of Premorbid Functioning provides a standardised approach for estimating an individual's level of cognitive and memory functioning before the onset of illness or injury. Individuals are given a list of words with atypical grapheme-to-phoneme translations. Thus, to pronounce them correctly an individual must have prior knowledge of the words.

The Momentary Influences, Attitudes and Motivation Impact on Cognitive Performance Scale captures the impact of motivation, momentary influences and test anxiety on neurocognitive performance. A preversion is

self-administered prior to starting a formal neuropsychological assessment and then a postversion is similarly self-administered at the conclusion of the formal assessment. A 20-item questionnaire captures four domains: poor motivation, concerns about assessment, fears about poor outcome and negative momentary influences.

Innate and adaptive immune response

We will conduct a global assessment of immune subsets (T cells, B cells, NK cells, iNKT cells, monocyte/macrophage) to determine subsets and activation status as a method to understand which immune arms are engaged in the response to LD. To this end, we will isolate PBMCs from blood specimens collected at baseline, 3 and 6 months. We will measure cellular immunity using multiparametric flow cytometry assays. We will be tracking changes in the T cell, B cell and NK cell compartments using a single 40-marker panel that will be run on a 5-laser Cytek Aurora. This panel captures a broad range of developmental and functional phenotypes, including CD4+T cell subsets, isotype-specific B cells and iNKT cells. The high-dimensional dataset will be analysed using peer-reviewed algorithms developed specifically for high-dimensional datasets. Our workflow stresses the proper preparation of spectral flow cytometry data for high-dimensional analysis and tools for integrating new data at later time points. The workflow includes components for quality control, data cleaning, transformation, batch effect correction, subsampling, clustering and data integration. The Bioconductor package flowAI facilitates the detection and removal of anomalies, and cleans the data upstream of determining the ideal transformation parameters using flowVS that minimises and stabilises signal variance per-channel. A reference control specimen is included with every cytometry run to adjust for interexperimental variations and the data are batch-corrected using the CytoNorm package. Raw, processed and analysed data are stored in a SingleCellExperiment container, which efficiently allows us to produce visualisations of the data in the form of Uniform Manifold Approximation and Projection (UMAP) plots, as well as standard biaxial, histogram, dot, box and bar plots.

Gene and cytokine profiling

We will collect peripheral blood at baseline and at 1, 3, 6 and 12 months. All specimens will be collected and immediately processed. For gene expression profiling, blood will be placed in PAXgene tubes (Quigen) and RNA isolated according to PAXgene specifications. We will use P3 kits and the NextSeq 2000 system. RNA will be quantified by NanoDrop and integrity will be assessed by the Agilent TapeStation system. For cytokine profiling, blood will be used for quantifying cytokine profiles including levels of CCL2, CCL3, IL-1β, IL-6, IL-8, IL-10, TNF, IFN-α and adaptive TH1 (IFN-γ, CXCL9, CXCL10, IL-12p40, IL-12p70, CCL19) or TH17 (IL-17A, IL-17F, IL-17E/IL-25, IL-21, IL-22, IL-23, IL-27). We will use the Millipore Sigma kits and the Bio-plex 200 system.

Participant timeline

A summary of activities and timelines is provided in figure 1.

Sample size

We anticipate following up to 120 participants in the LD cohort (60 early localised, 30 early disseminated, 30 late disseminated) over the study period. Each study participant enrolled in the study will contribute at least six time points (baseline, 1, 3, 6, 9, 12 months). We anticipate that between 15 and 20% of cohort participants will have poor outcomes beyond 12 months of follow-up. Sample size calculations focus on the random effect model for change over time in the dependent variables. Power and sample size calculations are not straightforward, but simplified calculations indicate that this sample size will be adequate for the analyses. For example, SF-36 scores are scaled from 0 to 100 points. Assuming a high SD of 20 points, moderate test–retest reliability for the SF-36 of 0.75, and adopting a type-1 error rate of 0.05, with 60 participants in the early localised category and 30 in the early disseminated category and 30 in the late disseminated category we will have 80% power to detect a change between two assessments of as little as 4.6 points. Enrolling 120 LD participants will give us the data we need to address the objectives of the study particularly since the number of covariates is limited. The rationale for the healthy controls are to provide a basis for comparison with the LD cohort for the immunological assessments. Given the challenge involved in recruiting healthy controls willing to be followed longitudinally, we believe that enrolling 30 is a number that can be achieved and at the same time provide a basis for comparison.

Given that we do not have pilot data on estimates of the innate immunity and T cell markers that we wish to assay in patients with LD, we have not attempted to calculate a sample size on this basis as we believe it would be speculative. However, we have done previous studies including patients with SARS, West Nile and dengue such that a sample of >100 patients should be sufficient to look at within patient (ie, different time points) and between patient differences in innate and adaptive immunity as well as RNA profiling.

Patient and public involvement

Patients and those with lived experience were involved in the design and conduct of this research. During the design of the study, patients informed the research questions, choice of outcome measures and methods of recruitment.

Recruitment and study status

Participants are being enrolled from emergency departments (EDs) and urgent care clinics in Kingston and Lunenburg by physicians at both study sites. Enrolment began on 31 May 2021, and 42 participants with LD along with 31 controls have been enrolled as of 7 September

	Acute Onset & Initiation of Treatment-Seeking	LD Cohort Permission to Contact	Healthy Control Cohort Advertise	Eligibility & Consent	Baseline (Visit 1)	1 month 30 days (V2)	3 months 90 days (V3)	6 months 180 days (V4)	9 months 270 days (V5)	12 months 360 days (V6)	24 months 720 days (V7)
Location		ED or Urgent Care	n/a	TBD	Ambulatory Care Setting						
Contact											
Script for ED physicians		✓									
Basic demographics Log		✓									
LD Cohorts - Assess Elibility/Obtain Informed Consent											
Screening Form				✓							
Informed Consent											
Healthy Control Cohort - Recruit, Assess, Consent											
Recruitment Poster			✓								
Eligibility Assessment				✓							
Informed Consent				✓							
LD Risk Factor Assessment											
Medical Release Form				✓							
Clinical Questionaire					✓	✓	✓	✓	✓	✓	✓
Economic Questionnaire					✓			✓		✓	✓
Diagnostic Testing: *(estimated testing time)*					3 hrs	45 min	2.5 hrs	3 hrs	45 min	3 hrs	3 hrs
SF-36 *(8-10 Min)*					✓	✓	✓	✓	✓	✓	✓
DASS *(5-10 Min)*					✓	✓	✓	✓	✓	✓	✓
FSS *(5 Min)*					✓	✓	✓	✓	✓	✓	✓
NeuroPsych Tests *(75 min)*					✓		✓	✓		✓	✓
Immunulogical Testing:											
PBMC					✓		✓	✓			
Gene & cytokine profiling					✓	✓	✓	✓		✓	
Laboratory Assessments											
Punch biopsy	Cohort participants may be approached by pillar 1 (diagnostics) under separate consent.										
ECG											
Serology (Biochemistry, acute phase reactants, Lyme IgM and IgG, PBMCs)		Tests that are normally ordered by a treating physician. Data to be obtained from participant medical records									
CSF	Depends on clinical presentation										
Joint Fluid	Depends on clinical presentation										

Figure 1 Summary of activities. CSF, Cerebrospinal fluid; DASS, Depression and Anxiety Severity Scale; ED, emergency department; FSS, Fatigue Severity Scale; LD, Lyme disease; PBMC, peripheral blood mononuclear cell; SF-36, Short-Form 36; TBD, to be determined.

2023. We anticipate that data collection will be complete by September 2025.

Analyses

Physical and mental functioning

Prognostic curves

We will use non-linear mixed-effects modelling to estimate the parameters of non-linear models for PCS, MCS, DASS and the FSS. We will compare those with early localised (ie, physician diagnosed EM), early disseminated, and late disseminated LD. We will describe change over time in neurocognitive testing for five key domains using seven tests with eight dependent variables: verbal learning and memory (HVLT-R total recall, delayed recall), visual learning and memory (BVMT-R total recall, delayed recall), executive functioning (TOL-execution time, problem solving time), attention (SCWT interference score) and speed of information processing (SDMT). Using random effects modelling, we will test the hypothesis that pre-existing comorbidity and severity of neurological illness at presentation are associated with worse long-term neuropsychological outcomes.

Proportional hazards models

We will construct Cox proportional hazards models to assess factors associated with time to normalisation of PCS and MCS scores. Factors to be assessed will include demographic factors, early antibiotic therapy (measured in days from date of onset of symptoms), comorbidity, cytokine profiles and immune phenotypes. We will specifically aim to assess whether early antibiotic therapy (measured in days from date of onset of symptoms) reduces time to recovery, and whether comorbidity on enrolment is important. For each component summary measure, we will model the number of days until normalisation of the score by type of LD (early localised, early disseminated, and late disseminated LD) and by the presence of comorbid conditions at enrolment. We will define days to normalisation from date of enrolment until the date of a score of 50 or more on the PCS or MCS. Participants who did not achieve a score of ≥50 will be censured. We will create time-dependent covariates to test the assumption of proportional hazard.

Innate and adaptive immune response

Unsupervised clustering will be performed by FlowSOM to identify meta-clusters within the dataset and assign cell types (eg, T cell, B cell, NK cell). These clustering results will form the basis for our statistical analyses using General Linear Mixed Modelling and Bioconductor packages, cytoGLMM and diffcyt, to report differential

functional states and cell population abundances in a temporal manner and relate the states and populations to participant outcomes.

Gene expression profiling

Following quality control procedures (eg, removal of outlier patterns or sex discrepancy) and normalisation and log-transformation, the data will be analysed using Student's t-test to assess changes in gene expression between LD and control groups. We will stratify the analysis by the type of LD patient (early localised, early disseminated, late disseminated).

ETHICS AND DISSEMINATION

The protocol has been approved by the research ethics boards at McMaster University (Hamilton Integrated Research Ethics Board) (7564), Queens University (EMD 315-20) and Nova Scotia Health Research Ethics Board (1027173). Written informed consent is obtained from all participants. Any changes to the protocol are submitted to these boards and must be approved prior to their implementation. Treating physicians will approach patients to see if they are willing to be contacted about the study by research staff. A research associate or nurse then assesses patient eligibility and obtains informed consent. The findings from this study will be disseminated locally and internationally through manuscript publications in peer-reviewed journals and conference presentations at national and international platforms. We will also include a lay summary of the findings for decision-makers and patients.

DISCUSSION

This cohort study of patients with LD will include a comprehensive long-term assessment of physical and mental functioning, including comprehensive neuropsychological testing.

Although there have been previous studies, there is a still a need for prospective cohort studies. In a cohort study of 74 participants over a period of 6 months, eight (11%) participants met an operationalised definition of post-treatment LD syndrome, including self-reported fatigue, musculoskeletal pain or cognitive complaints, and functional impact.[18] Our study aims to further refine physical and mental measures using standardised instruments. A retrospective cohort study of 38 participants with LD had more clinical symptoms (arthalgias, paresthesias) as well concentration difficulties and fatigue compared with controls.[19] In another retrospective study, participants with LD were more likely to have joint pain and symptoms of memory impairment as well as poor functional status than those without LD, while performance on neurocognitive tests did not differ.[20] A case series of 212 participants used self-reported symptoms for factor classification into three groups including fatigue—cognitive, musculoskeletal pain and mood.[21] We aim to build on these studies using the SF-36 for physical and mental functioning, as well as the DASS, FSS, and neurocognitive testing.

There have been relatively few longitudinal immunological assessments of LD. One cohort study of 38 participants with LD and 18 controls reported that plasmablasts were a key B cell population associated resolution of LD.[22] Several studies have assessed transcriptional profiles. In a study of 39 participants with disseminated disease and 23 controls, the transcriptome was dominated by INF-regulated genes reported during early convalescence while after 6 months profiles were similar to controls.[23] In another study of 29 LD patients and 13 controls, early LD prior to antibiotic therapy was characterised by marked upregulation of Toll-like receptor signalling but lack of activation of the inflammatory T cell apoptotic and B cell developmental pathways seen in other acute infectious syndromes.[24] Six months after completion of therapy, LD patients were found to have 31–60% of their pathways in common with three different immune-mediated chronic diseases. No differential gene expression signature was observed between LD patients with resolved illness to those with persistent. Another study followed 73 acute LD patients and uninfected controls over a period of a year and reported that RNA sequencing applied to PBMCs separated cases from controls, and almost all cases never return to cluster with the controls over time.[25] Assessment of cytokine patterns has also been limited. In a cohort study of 44 LD cases and 23 controls, a cytokine signature associated with the early stages of infection was identified and also delineated two subsets of acute LD patients with distinct cytokine signatures that differentiated symptom presentation.[26] Levels of the T cell chemokines CXCL9 (MIG), CXCL10 (IP-10) and CCL19 (MIP3B) were increased in one subgroup and this was associated with seroconversion status and elevated liver function tests.

Our study will also include a comprehensive immunological assessment of LD. Our cohort study will provide a global immunological assessment of LD including cellular immune analysis. This will allow for a correlation of immune functioning and mental and physical functioning. The study was specifically designed to help address questions important to patient groups and those with lived experience.

Despite the strengths of our study, there are limitations. Participation in the study requires willingness to undertake a large amount of testing. It could be that participants who are willing to participate differ from those who are unwilling or unable. We will assess differences in participants screened for demographic differences. To enrol participants, we will be flexible in terms of timing for measurement. Many participants will not want to spend additional hours in the ED answering research surveys and so we will allow participants to return the following day.

In conclusion, we anticipate that the findings of this study will make important contributions to knowledge about the natural history of LD.

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Contributors ML wrote the initial draft of the manuscript; ML, RB, JB, TH, BS designed the protocol; RB and ES are responsible for recruitment and retention of participants; JB for immunological investigations; all reviewed and edited the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by McMaster University (Hamilton Integrated Research Ethics Board) (7564), Queens University (EMD 315-20), and Nova Scotia Health Research Ethics Board (1027173). Participants gave informed consent to participate in the study before taking part.

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