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TITLE

Symptom Heterogeneity and Patient Subgroup Classification in a Clinical Case Series of Patients with Post-Treatment Lyme Disease

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ABSTRACT

Objectives: To identify underlying subgroups with distinct symptom profiles, and to characterize and compare these subgroups across a range of demographic, clinical, and psychosocial factors, within a heterogeneous group of patients with well-defined post-treatment Lyme disease.

Design: A clinical case series of patents.

Setting: Participants were recruited from a single-site, Lyme disease referral clinic patient population and were evaluated by physical exam, clinical laboratory testing, and standardized questionnaires.

Participants: Two hundred and twelve participants met study criteria for post-treatment Lyme disease, with medical record-confirmed prior Lyme disease as well as current symptoms and functional impact.

Results: Exploratory factor analysis classified 30 self-reported symptoms into six factors: "Fatigue Cognitive," "Ocular Disequilibrium," "Infection-Type," "Mood-Related," "Musculoskeletal Pain," and "Neurologic." A final latent profile analysis was conducted using "Fatigue Cognitive", "Musculoskeletal Pain", and "Mood-Related" factor-based scores, which produced three emergent symptom profiles, and participants were classified into corresponding subgroups with 59.0%, 18.9%, and 22.2% of the sample, respectively. Compared to the other two groups, subgroup 1 had similarly low levels across all factors relative to the sample as a whole, and reported lower rates of disability and higher self-efficacy. Subgroup 2 had the highest "Musculoskeletal Pain" factor-based scores, and had higher blood pressure as well as more abnormal C-reactive protein results. Subgroup 3 was characterized overall by higher symptom factor-based scores, and was found to be younger, to have a longer illness duration, and reported higher depression.

Conclusions: This analysis identified six symptom factors and three potentially clinically relevant subgroups among patients with well-characterized post-treatment Lyme disease. We found that these subgroups were differentiated not only by symptom phenotype, but also by a range of other factors. This may serve as an initial step towards engaging with the symptom heterogeneity that has long been observed among patients with this condition.

Keywords: Lyme disease, post-treatment Lyme disease, symptoms, patient subgroups

ARTICLE SUMMARY

Strengths and limitations of this study

- We operationalized a rigorous definition of post-treatment Lyme disease in our sample population, which ensured greater specificity of our findings to patients whose current illness is more evidently linked to prior Lyme disease.
- This specificity, and the regional focus of our sample population, may limit generalizability to the larger population of patients with persistent symptoms following treatment for Lyme disease, or those from other regions of the US.
- Reproducibility of the subgroup analysis may be affected by necessary methodological decisions incorporating statistical and clinical criteria which were made during the analytic process.
- We were able to draw upon a relatively large sample size of participants with well-characterized post-treatment Lyme disease, which allowed for clear and concise interpretability of data.

INTRODUCTION

Lyme disease is a tick-borne disease of increasing public health importance found primarily across temperate regions of the Northern Hemisphere.[1,2] Clinical signs of early infection may include a round, red, skin lesion occurring at the site of the bite of infected *Ixodes* ticks, and/or a transient, viral-like illness consisting of fever, fatigue, myalgia, or arthralgia.[1,3] If not promptly identified or otherwise left untreated, the bacteria (*Borrelia burgdorferi* in the United States) can disseminate to other areas of the skin, and via the blood stream to other organs such as the nervous system, heart, and joints.[4] Consequently, although less commonly observed, patients with untreated infection can present with objective, later manifestations of neurologic disease, carditis, or arthritis.[3]

While the majority of patients treated appropriately for Lyme disease recover, a subset develop a poorly-understood, chronic illness of persistent or recurrent symptoms following treatment.[5] In order to methodically advance scientific understanding, a standardized, highly-specific, research definition for post-treatment Lyme disease syndrome (PTLD, alternatively previously called post-Lyme disease syndrome or post-treatment Lyme disease) has been used

and operationalized to identify a subset of these patients with on-going symptoms linked temporally to strong evidence of prior exposure to *B. burgdorferi*.[6–8] The most prominent symptoms, and those included in the Infectious Diseases Society of America's (IDSA) proposed case definition of PTLD,[3] include fatigue, musculoskeletal pain, and cognitive dysfunction. However, patients with PTLD often also report a broad range of other neurologic, sleep, mood, viral-like, ocular, and other symptoms.[7,9,10] This heterogeneity is often compounded by the significant impact of these symptoms on patient quality of life and functioning.[7,11] Additionally, given the lack of: a) a sensitive and specific test to aid diagnosis, b) FDA-approved treatment options for patients, and c) a known etiology, PTLD presents a complex challenge to physicians.

As large studies among patients with well-characterized PTLD have not been conducted, this diversity in PTLD symptom reporting has not been comprehensively examined and it is unknown whether it may obscure the presence of distinct clinical patient subgroups. However, it is increasingly common that through advances in personalized medicine, diseases previously considered a single entity have been found instead to be comprised of clinically and/or biologically coherent subgroups.[12,13] Furthermore, similar to fibromyalgia, PTLD is likely a complex, multifactorial illness with immunologic, microbiologic, genetic, and/or psychosocial factors contributing to disease development, severity, and persistence.[5,14] Consequently, examining the heterogeneity of clinical presentations and symptom reporting that exists among patients with PTLD is important because it may inform a deeper understanding of etiology and effective treatment approaches. Therefore, the aims of this study were a) to identify underlying patient subgroups with distinct symptom profiles within a heterogeneous group of patients with well-defined PTLD, and b) to characterize and compare these subgroups across a range of demographic, clinical, laboratory, and psychosocial factors.

METHODS

Study Participants

Participants were recruited from a referral-based clinic population. Detailed recruitment information and enrollment criteria for this study were included in an initial publication describing a subset of the larger sample of participants included in the current analysis.[7] In brief, we replicated much of the criteria set forth in the IDSA's proposed case definition for

PTLD through our eligibility criteria.[3,7] Participants were required to have prior evidence in their medical record of appropriately treated, CDC-definite or probable Lyme disease.[15] They were also required to have current, functionally-impairing fatigue, pain, and/or cognitive dysfunction, and were excluded for a range of specific co-morbid medical conditions, as previously described.[7] For the current analysis, we did not limit the sample to those with greater than six month's illness duration, and thus, we refer to our sample as meeting criteria for post-treatment Lyme disease (PTLD). The Institutional Review Board of the Johns Hopkins University School of Medicine approved this study, and written informed consent was obtained from all study participants.

Patient and Public Involvement

Patients and the public were not directly involved in the design, recruitment, or assessment of this study.

Data Collection Instruments

Participants were asked to self-administer a 36-item symptom questionnaire (PLQS) developed based on prior clinical and research experience among patients with PTLD.[7] Participants indicated both presence and severity over the past two weeks for each symptom (0=absent, 1=mild, 2=moderate, or 3=severe). Of the original 36 symptoms, we excluded the following, which occurred with low frequency in our sample and were not considered to be core symptoms of PTLD (the percent endorsed at a moderate or severe level): urination pattern change (9%), diarrhea (9%), sore throat (4%), drooping eyelid(s) (2%), Bell's palsy (1%), and tender lymph nodes (2%). Data from the remaining 30 symptoms provided the basis for the subgroup analyses described below (see Supplemental Table S1 for the complete list of symptoms).

Participants were also asked to self-administer a battery of additional questionnaires included in the current analyses. The Beck Depression Inventory-II is a 21-item depression metric which can be divided into 'Somatic' and 'Cognitive-Affective' subscales.[16,17] In order to avoid duplication with other variables in this analysis, only the 'Cognitive-Affective' subscale (BDI-C/A) was included, which has a total score of 0-48. Quality of life was measured by the Short-Form Health Survey, Version 2 (SF-36).[18] This 36-item metric can be summarized into Physical and Mental Component Scores (PCS and MCS, respectively), with a higher score

indicating higher quality of life. These scores can also be compared with the US population mean (50.0 ± 10.0). The Life Events Checklist (LEC) is a 17-item measure with total scores of 0-17 of prior potentially traumatic events originally developed to aid in the diagnosis of post-traumatic stress disorder.[19] The Stanford Chronic Disease Self-Efficacy Scale (CDSE) is a 6-item measure of perceived self-efficacy for chronic disease self-management.[20,21] The Big Five Inventory (BFI) is a 44-item measure of five personality dimensions; extraversion, agreeableness, conscientiousness, emotional stability, and openness.[22–24] Variables related to prior, initial Lyme disease clinical presentation, treatment(s), and duration of illness were abstracted from participants' medical records from the time of Lyme disease onset. Participants self-reported other prior medical diagnoses as part of a structured clinical interview.

During the study visit, a physical exam was performed which included routine measures of height, weight, pulse, and blood pressure. Body mass index (BMI) was calculated using the standard formula (weight [kg] / height [m^2]). Vibratory index was measured on the distal interphalangeal joint of the index finger and on the interphalangeal joint of the hallux using a Rydel-Seiffer 64 Hz tuning fork.[25] Lastly, participants underwent a blood draw, and standard clinical tests (CBC, CMP, C-reactive protein, and two-tier serology for antibodies to *B. burgdorferi*) were performed by a large, commercial laboratory.

Statistical Analysis

We first performed exploratory factor analysis (EFA) to identify the latent relational structure of the symptoms included in the PLQS, which subsequently also reduced the dimensionality of the data. The Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) and Bartlett's test of sphericity were used to check whether the data were suitable for factor analysis. Considering the ordinal nature of the variables, both polychoric and Pearson's correlation coefficients were used. We chose the minimal residual estimation method because it can be used when the sample size is relatively small and when the correlation matrix is non-positive definite.[26] Oblique rotation was used to allow for correlations between extracted factors. The number of retained factors was informed by the visual scree test and parallel analysis, while taking into consideration clinical meaningfulness and the balance between parsimony and comprehensiveness. We used a factor loading cutoff value of 0.3.

Next, to uncover subgroups of participants we performed latent profile analysis (LPA) on the standardized symptom factor-based scores generated by the EFA. The number of identified clusters was determined based on minimization of the Bayesian information criteria (BIC) and the correlational structure of the data. Lastly, pairwise sub-group differences were examined and summarized using 2-sample t test or Wilcoxon rank sum test for continuous variables and chi-squared or Fisher's exact test for categorical variables.

A p-value less than 0.05 was considered significant. All statistical analyses were performed using R (version 3.6.1).

RESULTS

Participant Characteristics

A total of 225 participants with PTLD were enrolled in the study. We excluded six participants whose PTLD symptoms began more than six months after their initial Lyme disease episode, and seven participants who missed all symptom variables on the PLQS, for a total of 212 in the final sample. We employed mean imputation for three participants who each missed one of the 30 PLQS variables included in the analysis. Table 1 shows a description of the final participant sample. The average age was 48 years and there was a slight (58.5%) majority male in the sample. A large majority were residents of Mid-Atlantic states at the time of their disease onset (93.4%) and/or residents of states considered 'high-incidence' for Lyme disease (96.7%).[27]

Table 1. Characteristics of 212 participants with well-defined post-treatment Lyme disease[a]

	All Participants n = 212
Age at study visit	48.00 [37.00, 58.00] (18.00, 82.00)
Male gender	124 (58.5%)
White, non-Hispanic	190 (89.6%)
Years of education	16.00 [14.00, 18.00] (10.00, 30.00)
Annual household income >$100K	119/203 (58.6%)
Currently out of work on disability	12 (5.7%)

Lyme disease onset while resident of CDC Lyme disease 'high-incidence' state[27]	205 (96.7%)
CDC 'confirmed' initial Lyme disease presentation[15]	124 (58.5%)
Duration of illness from onset of PTLD symptoms to study visit (years)	1.67 [0.68, 3.81] (0.06, 28.59)
Total antibiotic exposure from symptom onset (weeks)	8.57 [4.43, 14.29] (2.00, 168.57)

[a]Data from categorical variables are presented as count (%). Data from normally distribute variables are presented as mean ± standard deviation (range) and from continuous variables without normal distribution as median [25th percentile, 75th percentile] (range). Proportions were calculated based on non-missing data and may not add to 100% because of rounding. Missing data are as follows: Years of education, 1 (0.5%); Annual household income, 9 (4.2%).

Latent Relational Structure among Symptoms

In the EFA analysis, the original polychoric correlation matrix was non-positive definite. After smoothing was performed to arrive at a positive definite matrix, it resulted in a poor overall sampling adequacy index (0.10) and an ultra-Heywood case was detected. However, the overall measure of sampling adequacy based on the Pearson's correlation coefficient was 0.86 (meritorious), and Bartlett's test of sphericity was significant ($p<0.001$). A 6-factor model was suggested by both statistical criteria and clinical meaningfulness (Figure 1, see Supplemental Table S1 for the complete factor pattern matrix). The root mean square of the residuals was 0.04, the root mean square error of approximation index was 0.06, and the Tucker Lewis index of factoring reliability was 0.85. The symptoms headache, poor coordination, and lower back pain were removed due to close cross loading (difference less than 0.10) across two factors. The percent endorsed at a moderate or severe level for these symptoms was 15.6%, 4.2%, and 9.4%, respectively. An expert physician on the study team (JA) named the factors as "Fatigue Cognitive," "Ocular Disequilibrium," "Infection-Type," "Mood-Related," "Musculoskeletal Pain," and "Neurologic." All six factors were weakly or moderately correlated with each other (0.21 to 0.41), with the strongest correlation between the "Fatigue Cognitive" and "Mood-Related" factors. Six factor-based scores were calculated for each participant by adding up the scores of the symptoms within each factor, and then these factor-based scores were standardized to have a mean of zero and a standard deviation of one.

Participant Subgroup Analysis

For the LPA analysis, we did not include the "Ocular Disequilibrium" factor as it prevented the LPA from converging for most of the specified models in model selection, possibly due to its low endorsement rate (the percentage endorsing symptoms included in this factor at a moderate or severe level ranged from 0.9% to 24.1%). When conducted on the remaining five factors, LPA classified participants into two groups based on their overall level of symptom reporting (high vs. low) relative to the sample as a whole.

We then conducted a secondary LPA incorporating those factors which contained only the most common PTLD-defining symptoms as well as mood (i.e. "Fatigue Cognitive", "Musculoskeletal Pain", and "Mood-Related"). Three symptom profiles emerged (Figure 2) and participants were classified into subgroups corresponding to these symptom profiles. Subgroup 1 contained 59.0% of the participants and was characterized by similarly low levels across all three factors relative to the sample as a whole. Subgroups 2 and 3 contained 18.9% and 22.2% of the participants, respectively, and were characterized by overall higher levels of the three factors relative to the entire sample. These results remained stable when the "Neurologic" factor was re-introduced in the LPA.

Participant Subgroup Comparisons

We first compared the three subgroups generated by the LPA across all six original PLQS factor-based symptom scores (Figure 3). Compared to subgroup 1, "Fatigue Cognitive" and "Neurologic" factor-based scores were significantly higher among both subgroup 2 and 3 participants. "Musculoskeletal Pain" was the only factor to statistically significantly differentiate all three subgroups from one another, with increasing scores from subgroup 1 to 3, however "Infection-Type" and "Ocular Disequilibrium" factor scores also trended in that direction. Lastly, "Mood-Related" factor scores were significantly higher among subgroup 3 participants compared both to subgroups 1 and 2, which did not differ significantly from each other.

Results of detailed demographic, clinical, laboratory, and psychosocial characteristic comparisons by subgroup are presented in Table 2. Notably, neither the percentage male (p>0.703 for all pair-wise comparisons) nor LEC total score (p>0.331 for all pair-wise comparisons) were statistically significantly different across subgroups. Participants in subgroup 1, which generally included those with lower symptom factor-based scores, also reported lower rates of being on disability than the other two groups and had higher CDSE scores. Subgroup 2

was found to have higher blood pressure, and a higher percentage of participants with an abnormal C-reactive protein than the other two subgroups.

Table 2. Patient subgroup comparisons across demographic, clinical laboratory, and psychosocial characteristics[a]

	Subgroup 1 *n*=125	Subgroup 2 *n*=40	Subgroup 3 *n*=47	*p*-value 1 vs. 2	*p*-value 1 vs. 3
DEMOGRAPHIC					
Age at study visit (years)	49.00 [40.00, 61.00] (18.00, 82.00)	51.00 [40.75, 56.00] (25.00, 70.00)	42.00 [27.00, 52.00] (18.00, 82.00)	0.729	0.032
Male gender	75 (60.0%)	23 (57.5%)	26 (55.3%)	0.924	0.703
White, non-Hispanic	111 (88.8%)	34 (85.0%)	45 (95.7%)	0.717	0.240
Years of education	16.00 [14.00, 18.00] (10.00, 25.00)	16.00 [14.00, 18.00] (12.00, 30.00)	16.00 [14.25, 18.00] (12.00, 22.00)	0.842	0.718
Annual household income >$100K	78/117 (66.7%)	23 (57.5%)	18/46 (39.1%)	0.393	0.002
Out of work on disability	2 (1.6%)	4 (10.0%)	6 (12.8%)	0.031	0.006
Body mass index (kg/m^2)	25.72 [22.71, 29.42] (16.47, 38.88)	26.78 [22.59, 30.50] (19.80, 41.74)	26.15 [23.47, 29.29] (18.99, 45.70)	0.331	0.564
CLINICAL/PHYSICAL EXAM					
Illness duration from disease onset to study visit (years)	1.45 [0.59, 3.84] (0.15, 28.59)	1.30 [0.71, 2.14] (0.06, 13.13)	2.23 [1.03, 5.56] (0.13, 18.67)	0.431	0.101
CDC 'confirmed' initial Lyme disease[15]	77 (61.6%)	21 (52.5%)	26 (55.3%)	0.404	0.566
Initial late Lyme arthritis	15 (12.0%)	3 (7.5%)	1 (2.1%)	0.566	0.073
Initial neurologic Lyme disease	7 (5.6%)	2 (5.0%)	7 (14.9%)	1.000	0.094
Time to initial recommended antibiotic treatment (days)[b]	23.00 [0.00, 110.00] (0.00, 10000.00)	14.50 [0.00, 181.25] (0.00, 757.00)	14.00 [2.50, 128.00] (0.00, 3700.00)	0.692	0.723
Total antibiotic exposure since disease onset (weeks)	8.00 [4.43, 13.00] (2.00, 112.86)	7.64 [4.29, 19.21] (3.00, 130.00)	9.00 [5.64, 14.71] (2.86, 168.57)	0.813	0.270
Intravenous antibiotic use	26 (20.8%)	7 (17.5%)	20 (42.6%)	0.820	0.007
Non-recommended antibiotic exposure prior to recommended antibiotic exposure[b]	17 (13.6%)	4 (10.0%)	8 (17.0%)	0.786	0.745
Steroid exposure after disease onset, prior to recommended antibiotic treatment[b]	10 (8.0%)	7 (17.5%)	4 (8.5%)	0.155	1.000
Systolic blood pressure (mmHg)	125.50 [114.00, 137.50] (92.00, 171.00)	133.00 [121.75, 144.25] (106.00, 173.00)	126.00 [115.00, 138.00] (99.00, 179.00)	0.021	0.848
Diastolic blood pressure (mmHg)	80.82 ± 9.36 (63.00, 103.00)	85.53 ± 9.34 (64.00, 110.00)	82.47 ± 8.93 (63.00, 100.00)	0.007	0.300
Pulse (beats per minute)	68.00 [61.50, 73.00] (48.00, 120.00)	70.50 [64.00, 81.00] (52.00, 106.00)	70.00 [64.00, 80.25] (51.00, 104.00)	0.052	0.165
Vibratory sense abnormal[c]	34/124 (27.4%)	15/39 (38.5%)	10/45 (22.2%)	0.266	0.630
CO-MORBIDITIES					
Thyroid disease	9 (7.2%)	4 (10.0%)	4 (8.5%)	0.518	0.753

Heart disease or Hypertension	20 (16.0%)	5 (12.5%)	7 (14.9%)	0.800	1.000
Migraine headaches	17 (13.6%)	10 (25.0%)	18 (38.3%)	0.147	0.001
Carpal tunnel syndrome	13 (10.4%)	5 (12.5%)	4 (8.5%)	0.772	1.000
Neuropathy/neuromuscular disorder	8 (6.4%)	3 (7.5%)	6 (12.8%)	0.729	0.295
LABORATORY					
Absolute lymphocyte count ($10^3/\mu L$)	1.96 [1.56, 2.19] (0.68, 3.82)	1.89 [1.59, 2.26] (1.09, 4.29)	1.87 [1.63, 2.29] (0.82, 3.26)	0.954	0.412
C-reactive protein abnormal	6/119 (5.0%)	8/38 (21.1%)	3/43 (7.0%)	0.007	0.701
Reactive IgG bands on two-tier testing for antibodies to *B. burgdorferi*	5.00 [2.00, 8.00] (0.00, 10.00)	4.00 [2.00, 7.00] (0.00, 10.00)	4.00 [2.00, 6.50] (0.00, 10.00)	0.434	0.154
PSYCHOSOCIAL					
Beck Depression Inventory-II Cognitive/Affective subscale score[17]	5.00 [1.00, 8.00] (0.00, 20.00)	6.00 [4.00, 8.00] (0.00, 17.00)	13.00 [9.00, 19.00] (3.00, 39.00)	0.243	< 0.001
Stanford Chronic Diseases Self-Efficacy total score[20,21]	7.50 [5.30, 8.50] (1.00, 9.80)	6.00 [4.30, 7.55] (1.00, 9.80)	5.30 [4.25, 6.80] (1.00, 9.70)	0.014	< 0.001
Life Events Checklist total score[19]	2.00 [1.00, 4.00] (0.00, 13.00)	2.00 [0.00, 3.25] (0.00, 8.00)	2.00 [0.50, 4.00] (0.00, 9.00)	0.331	0.621
Big Five Inventory: Extraversion score[23]	3.38 [2.75, 3.88] (1.38, 5.00)	3.44 [3.00, 3.91] (1.63, 4.88)	3.13 [2.56, 3.63] (1.75, 5.00)	0.500	0.234
Big Five Inventory: Agreeableness score	4.00 [3.67, 4.44] (2.44, 5.00)	4.22 [3.97, 4.56] (2.33, 5.00)	3.89 [3.38, 4.38] (2.33, 5.00)	0.095	0.175
Big Five Inventory: Conscientiousness score	4.00 [3.56, 4.44] (2.22, 5.00)	4.05 [3.67, 4.44] (2.22, 4.89)	3.67 [3.28, 4.11] (1.56, 4.89)	0.747	0.003
Big Five Inventory: Emotional Stability score	3.63 [3.13, 4.10] (1.38, 5.00)	3.75 [3.22, 4.25] (2.50, 5.00)	2.63 [1.82, 3.25] (1.00, 4.63)	0.371	< 0.001
Big Five Inventory: Openness score	3.70 [3.30, 4.20] (2.30, 5.00)	3.90 [3.40, 4.32] (2.70, 4.90)	3.80 [3.30, 4.10] (1.20, 4.80)	0.216	0.997

[a]Data from categorical variables are presented as count (%). Data from normally distribute variables are presented as mean ± standard deviation (range) and from continuous variables without normal distribution as median [25th percentile, 75th percentile] (range). Proportions were calculated based on non-missing data and may not add to 100% because of rounding. Missing data are as follows: years of education, 1 (0.5%); annual household income, 9 (4.2%); body mass index, 18 (8.5%); systolic blood pressure, 5 (2.4%); diastolic blood pressure, 4 (1.9%); pulse, 3 (1.4%); vibratory sense, 4 (1.9%); absolute lymphocyte count, 2 (0.9%); C-reactive protein, 12 (5.7%); IgG reactive bands, 1 (0.5%); Beck Depression Inventory-II Cognitive/Affective score, 1 (0.5%); Stanford Chronic Diseases Self-Efficacy score, 1 (0.5%); Big Five Inventory, 3 (1.4%). [b]Recommended antibiotic regimens were considered any of the following: Doxycycline 100mg BID for ≥ 10 days, Tetracycline 500mg TID for ≥ 14 days, Ceftin 500mg BID for ≥ 14 days, Ceftriaxone 2g Q24 ≥ 14 days. Other drugs, or lower doses or durations were considered non-recommended antibiotic regimes. [c]Below age-adjusted normal vibration threshold values in either upper (distal interphalangeal joint of the index finger) or lower (interphalangeal joint of the hallux) extremities on either right or left side using a Rydel-Seiffer 64 Hz tuning fork.[25]

Overall, participants in subgroup 3 were younger, with a lower percentage reporting an annual household income > $100,000. This group was also found to have a median illness

duration of almost a year longer than the other two groups, and a significantly higher percentage who reported prior IV antibiotic treatment. Consistent with the pattern of symptom reporting in the factor-based PLQS scores, subgroup 3 had significantly worse BDI-C/A scores than the other two subgroups. On the BFI, subgroup 3 had significantly lower scores in the Conscientiousness and Emotional Stability domains than the other two subgroups. Additionally, compared to subgroup 2, subgroup 3 also had lower scores in the Agreeableness domain.

Those co-morbid diagnoses occurring with at least 5% prevalence in the sample as a whole are also reported in Table 2. No statistically significant differences were found for any of the conditions with the exception that participants in subgroup 3 were almost three times as likely as those in subgroup 1 to report migraine headaches. In examining differences by subgroup in SF-36 quality of life scores, we found that subgroup 2 had significantly lower PCS scores compared to the other two groups, whereas subgroup 3 had significant lower MCS scores compared to the other two groups (Figure 4). This is consistent with the pattern of symptom reporting in the factor-based scores which differentiated the three groups.

DISCUSSION

PTLD is a complex illness which is characterized by a wide range of clinical symptoms that can significantly impact quality of life for many patients.[7,9–11] The aim of this study was to examine heterogeneity in symptom reporting in order to ultimately identify and characterize clinically relevant patient subgroups. Using our PLQS questionnaire, we first identified six symptom-based factors through EFA analysis. The relational structure of these results had overall clinical face validity, with symptoms clustering in seemingly physiologically relevant rather than randomly distributed ways. For example, all three cognitive symptoms loaded onto the same factor, as did joint pain, muscle pain, and joint swelling. Furthermore, the six factors we identified represent commonly recognized domains in the clinical phenotype of PTLD.

Although the analyses and the measure differed, results from our EFA were generally consistent with those from a recent study with some participant sample overlap, which aimed to validate the General Symptom Questionnaire-30 (GSQ-30) in PTLD.[28] One noticeable difference was that fatigue loaded with the musculoskeletal pain factor in the GSQ-30 study rather than with cognitive symptoms, as it did in the current study. This suggests that fatigue in PTLD could arise from multiple sources including pain, the central nervous system, or muscle

weakness. Similarly, insomnia may also be a multifactorial symptom, as it showed low loading (0.32) to the 'Infection-Type' factor in the current study, with significant cross-loading to the 'Fatigue Cognitive', 'Musculoskeletal Pain', and 'Mood-Related' factors.

Several additional symptom factor loadings were informative as well. Neck pain is relatively common in the general population,[29] however it is reported with greater frequency and severity in this sample population compared to controls,[7] and the cause is unknown. Given that neck pain loaded the strongest onto the 'Neurologic' factor, with the second strongest loading to 'Fatigue Cognitive' and not 'Musculoskeletal Pain', we hypothesize the potential for a neurologic rather than arthritic origin. We also found that difficulty breathing and heart palpitations loaded onto the 'Mood-Related' factor, implying that this constellation of symptoms may result from a common pathway such as autonomic nervous system activation or central sensitization[30] rather than specific cardiac or pulmonary pathology. Alternatively, anxiety and other mood-related symptoms could result secondary to experiencing these types of distressing physiologic symptoms. The hypothetical relational constructs we uncovered using EFA may shed light on, but not necessarily equate to, distinct biological mechanisms resulting in symptoms. Some symptoms may have a composite underlying mechanism, some may correlate with each another despite different mechanisms, and some distinct factors could represent different sub-types of a shared general mechanism.

We then used a subset of the symptom-based factors in an LPA analysis to ultimately identify three patient subgroups corresponding to specific symptom profiles. This subgroup classification was prominently differentiated first by overall severity of symptom reporting, where high and low symptom reporters were identified. We plan to investigate factors associated with severity in the sample as a whole in future multivariate analyses. It is important to clarify that symptom severity in the current study is relative to this study sample of participants with PTLD and not the general population; we have previously shown a higher symptom burden in a subset of this sample of patients with PTLD compared to non-Lyme infected controls.[7]

Similar to our previous GSQ-30 study,[28] we conclude that morbidity in this population can exist above and beyond the effects of mood-related symptoms. Indeed, in our EFA analysis an independent "Mood-Related" factor was formed whose symptoms failed to load with other core symptoms of PTLD such as fatigue, pain, and cognitive difficulty. This is also supported by the pattern of symptom factor-based score reporting in subgroup 2. This subgroup had the

highest "Musculoskeletal Pain" factor-based scores, however their 'Mood-Related" factor-based scores remained relatively low, similar to those of subgroup 1. This pattern also suggests that mood-related symptoms in PTLD may be more likely to be associated with fatigue or cognitive symptoms than with pain. Moreover, although fatigue/cognitive, mood-related, and pain symptoms all formed discrete factors in our analysis, "Mood-Related" factor scores were more strongly correlated with "Fatigue Cognitive" than they were with "Musculoskeletal Pain" scores (0.41 vs. 0.21, respectively).

We did define a subset of our sample (22.2%, subgroup 3) who overall reported significantly higher "Mood-Related" factor-based scores relative both to the other two subgroups and to their other symptom factor-based scores. Comparing subgroups across a variety of domains suggests several possible explanations for this finding. First, despite being younger, participants in subgroup 3 had a longer illness duration, as abstracted from their medical record. We would hypothesize that the effects of a chronic, often functionally impairing illness on mood would both compound over time and be more pronounced among younger patients. Second, subgroup 3 also endorsed lower self-efficacy in managing their illness. This is unsurprising, as lower self-efficacy has been found to be associated with a higher degree of mood symptoms in a number of studies.[31,32] Furthermore, participants in subgroup 3 also scored lower on the Conscientiousness and Emotional Stability dimensions of the BFI, although additional research is warranted to explore the complex construct of personality among patients with PTLD. In sum, our findings suggest that participants in subgroup 3 may have been more psychologically vulnerable to the effects of a significant chronic illness over time when they first encountered Lyme disease. Indeed, many of the psychosocial variables that we measured have been shown to impact illness and resilience in other similar chronic disease populations.[33–35]

Finally, our data also suggest that participants with prior neurologic pathology may be over-represented in subgroup 3. Although the subgroup comparisons were not statistically significant, we observed that these participants had almost three times the rate of prior neurologic Lyme disease (cranial nerve palsy, neuropathy, meningitis or encephalitis), as abstracted from their medical record, compared to the other two groups. This is consistent with the higher rate of prior intravenous antibiotic treatment in this group as well. We also found that participants in subgroup 3 were significantly more likely to report a co-morbid diagnosis of migraines. In post-hoc analyses, the diagnosis of migraine predated the Lyme disease onset for

80% of those in subgroup 3 with migraine. It is possible that pre-existing neurologic vulnerabilities, such as a history of migraine and/or frank neurologic Lyme disease, are associated with a post-treatment phenotype that encompasses an increase in mood-related symptoms.[36] Although, per the IDSA case definition, we excluded participants with major psychiatric illness, Lyme disease has been associated with a range of neurologic and neuropsychiatric symptoms.[37] Strikingly, although female gender[38,39] and greater exposure to prior stressful life events[40] have both been associated with higher mood symptoms in a number of studies, we did not observe that these participants were any more likely to report heightened mood-related symptoms when faced with similar physical symptom levels.

Our study does have limitations. We ensured greater specificity of our findings to patients whose current illness is more evidently linked to *B. burgdorferi* exposure by operationalizing a narrow research definition of PTLD as eligibility criteria for inclusion into our sample. However, this specificity may also limit generalizability of our findings to a larger population of patients with persistent symptoms following treatment for Lyme disease, especially atypical early presentations not meeting CDC criteria. It is possible that different eligibility criteria, or different patient samples drawn from other regions of the United States, may have different results. Furthermore, we relied upon self-report symptom data for these analyses, which is subject to response bias as well as individual variation in perception of symptom severity.[41]

Finally, reproducibility of the subgroup analysis may be affected by necessary methodological decisions made during the analytic process, including; the scale of the data, the inclusion of a large number of symptoms in the analysis, and the statistical and clinical criteria used during the model selection process. However, the approaches we employed were chosen to achieve as high a degree of theoretical soundness and feasibility as possible. These approaches, in conjunction with the relatively large sample of participants with PTLD that we were able to draw upon for this analysis, allowed for clear and concise interpretability of data.

This analysis represents one of the first to identify and characterize potentially clinically relevant patient subgroups in PTLD. This is important as it may serve as an initial step towards engaging with the heterogeneity in symptom reporting that has long been observed among patients with this condition. Furthermore, in the future it may lead to more targeted interventions

or other novel treatment approaches to address the varied and/or multiple factors which contribute to illness perpetuation in PTLD.

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AUTHOR CONTRIBUTIONS

AR, TY, and JA all contributed to the conception and design of this study. TY and AR conducted the data management and statistical analyses. AR, TY, and JA drafted, revised, and gave final approval of the manuscript for publication.

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COMPETING INTERESTS

None to declare for any of the authors.

DATA SHARING

De-identified participant data are available upon reasonable request to the corresponding author.

FIGURES

Figure 1. Exploratory factor analysis of 30 common PTLD symptoms suggests a 6-factor model. Three of the symptoms did not load and were dropped in the final model.

Figure 2. Three subgroups of participants identified based on latent profile analysis (panels A and B).

Figure 3. Participant subgroup differences in median standardized symptom factor-based scores.

Figure 4. SF-36 health-related quality of life physical and mental component scores[18] for the three patient subgroups. ns = Not Significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

REFERENCES

1 Steere AC, Strle F, Wormser GP, *et al.* Lyme borreliosis. *Nat Rev Dis Prim* 2016;**2**:16090. doi:10.1038/nrdp.2016.90

2 Stone BL, Tourand Y, Brissette CA. Brave new worlds: the expanding universe of Lyme disease. Vector-Borne Zoonotic Dis. 2017;**17**:619–29. doi:10.1089/vbz.2017.2127

3 Wormser GP, Dattwyler RJ, Shapiro ED, *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**:1089–134. doi:10.1086/508667

4 Wormser GP. Hematogenous dissemination in early Lyme disease. *Wien Klin Wochenschr* 2006;**118**:634–7. doi:10.1007/s00508-006-0688-9

5 Rebman AW, Aucott JN. Post-treatment Lyme disease as a model for persistent symptoms in Lyme disease. *Front Med* 2020;**7**. doi:10.3389/fmed.2020.00057

6 Wormser GP. IDSA Treatment Guidelines for Lyme Disease : Panel Presentation. 2006.

7 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* 2017;**4**:224. doi:10.3389/fmed.2017.00224

8 Aucott JN. Posttreatment Lyme Disease Syndrome. *Infect Dis Clin North Am* 2015;**29**:309–23. doi:10.1016/j.idc.2015.02.012

9 Lobraico J, Butler A, Petrini J, *et al.* New insights into stages of Lyme disease symptoms from a novel hospital-based registry. *J Prim Care Community Heal* 2014;**5**:284–7. doi:10.1177/2150131914540693

10 Zomer TP, Barendregt JNM, van Kooten B, *et al.* Non-specific symptoms in adult patients referred to a Lyme centre. *Clin Microbiol Infect* 2019;**25**:67–70. doi:10.1016/j.cmi.2018.09.016

11 Klempner MS, Hu LT, Evans J, *et al.* Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *N Engl J Med* 2001;**345**:85–92. doi:10.1056/NEJM200107123450202

12 Mukherjee S, Mez J, Trittschuh EH, *et al.* Genetic data and cognitively defined late-onset Alzheimer's disease subgroups. *Mol Psychiatry* Published Online First: 4 December 2018. doi:10.1038/s41380-018-0298-8

13 Curtis C, Shah SP, Chin S-F, *et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;**486**:346–52. doi:10.1038/nature10983

14 Bellato E, Marini E, Castoldi F, *et al.* Fibromyalgia Syndrome: Etiology, Pathogenesis,

Diagnosis, and Treatment. *Pain Res Treat* 2012;**2012**. doi:10.1155/2012/426130
15 Centers for Disease Control and Prevention. Lyme disease (Borrelia burgdorferi) 2011 case definition. https://wwwn.cdc.gov/nndss/conditions/lyme-disease/case-definition/2011/
16 Beck AT, Steer RA, Brown GK. *Beck Depression Inventory - Second Edition Manual*. San Antonio: : The Psychological Corporation 1996.
17 Storch EA, Roberti JW, Roth DA. Factor structure, concurrent validity, and internal consistency of the Beck Depression Inventory-Second Edition in a sample of college students. *Depress Anxiety* 2004;**19**:187–9. doi:10.1002/da.20002
18 Ware JE, Kosinski M, Dewey JE. *How to score version 2 of the SF-36 health survey*. Lincoln, RI: : Quality Metric Inc. 2000.
19 Gray MJ, Litz BT, Hsu JL, *et al.* Psychometric properties of the life events checklist. *Assessment* 2004;**11**:330–41. doi:10.1177/1073191104269954
20 Lorig KR, Sobel DS, Ritter PL, *et al.* Effect of a self-management program on patients with chronic disease. *Eff Clin Pr* 2001;**4**:256–62.http://www.ncbi.nlm.nih.gov/pubmed/11769298
21 Ritter PL, Lorig K. The English and Spanish Self-Efficacy to Manage Chronic Disease Scale measures were validated using multiple studies. *J Clin Epidemiol* 2014;**67**:1265–73. doi:10.1016/j.jclinepi.2014.06.009
22 John OP, Naumann LP, Soto CJ. Paradigm shift to the integrative Big Five Trait taxonomy: History, measurement, and conceptual issues. In: John OP, Robins RW, Pervin LA, eds. *Handbook of personality: Theory and research*. New York, NY: : Guilford Press 2008. 114–58. doi:10.1016/S0191-8869(97)81000-8
23 John OP, Donahue EM, L KR. The Big Five Inventory--Versions 4a and 54. Berkeley, CA: 1991. doi:10.1016/S0092-6566(03)00046-1
24 Benet-Martínez V, John OP. Los Cinco Grandes Across Cultures and Ethnic Groups: Multitrait Multimethod Analyses of the Big Five in Spanish and English. *J Pers Soc Psychol* 1998;**75**:729–50. doi:10.1037/0022-3514.75.3.729
25 Martina IS, van Koningsveld R, Schmitz PI, *et al.* Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. *J Neurol Neurosurg Psychiatry* 1998;**65**:743–7.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2170371/pdf/v065p00743.pdf
26 Briggs NE, MacCallum RC. Recovery of weak common factors by maximum likelihood and ordinary least squares estimation. *Multivariate Behav Res* 2003;**38**:25–56. doi:10.1207/S15327906MBR3801_2
27 Centers for Disease Control and Prevention. Lyme Disease Maps: Most Recent Year. https://www.cdc.gov/lyme/datasurveillance/maps-recent.html (accessed 28 Aug 2019).
28 Fallon BA, Zubcevik N, Bennett C, *et al.* The General Symptom Questionnaire-30 (GSQ-30): a brief measure of multi-system symptom burden in Lyme disease. *Front Med* 2019;**6**:283. doi:10.3389/fmed.2019.00283
29 Safiri S, Kolahi A-A, Hoy D, *et al.* Global, regional, and national burden of neck pain in the general population, 1990-2017: systematic analysis of the Global Burden of Disease Study 2017. *BMJ* 2020;**368**:m791. doi:10.1136/bmj.m791
30 Batheja S, Nields JA, Landa A, *et al.* Post-treatment Lyme syndrome and central sensitization. *J Neuropsychiatry Clin Neurosci* 2013;**25**:176–86.

doi:10.1176/appi.neuropsych. 12090223

31 Muris P. Relationships between self-efficacy and symptoms of anxiety disorders and depression in a normal adolescent sample. *Pers Individ Dif* 2002;**32**:337–48. doi:10.1016/S0191-8869(01)00027-7

32 Shnek ZM, Foley FW, LaRocca NG, *et al.* Helplessness, self-efficacy, cognitive distortions, and depression in multiple sclerosis and spinal cord injury. Ann. Behav. Med. 1997;**19**:287–94. doi:10.1007/BF02892293

33 Galvez-Sánchez CM, Montoro CI, Duschek S, *et al.* Depression and trait-anxiety mediate the influence of clinical pain on health-related quality of life in fibromyalgia. *J Affect Disord* 2020;**265**:486–95. doi:10.1016/j.jad.2020.01.129

34 Torres X, Bailles E, Valdes M, *et al.* Personality does not distinguish people with fibromyalgia but identifies subgroups of patients. *Gen Hosp Psychiatry* 2013;**35**:640–8. doi:10.1016/j.genhosppsych.2013.07.014

35 Van Liew C, Leon G, Neese M, *et al.* You get used to it, or do you: symptom length predicts less fibromyalgia physical impairment, but only for those with above-average self-efficacy. *Psychol Heal Med* 2019;**24**:207–20. doi:10.1080/13548506.2018.1524152

36 Affaitati G, Costantini R, Tana C, *et al.* Co-occurrence of pain syndromes. *J Neural Trans* 2020;**127**:625–46. doi:doi: 10.1007/s00702-019-02107-8.

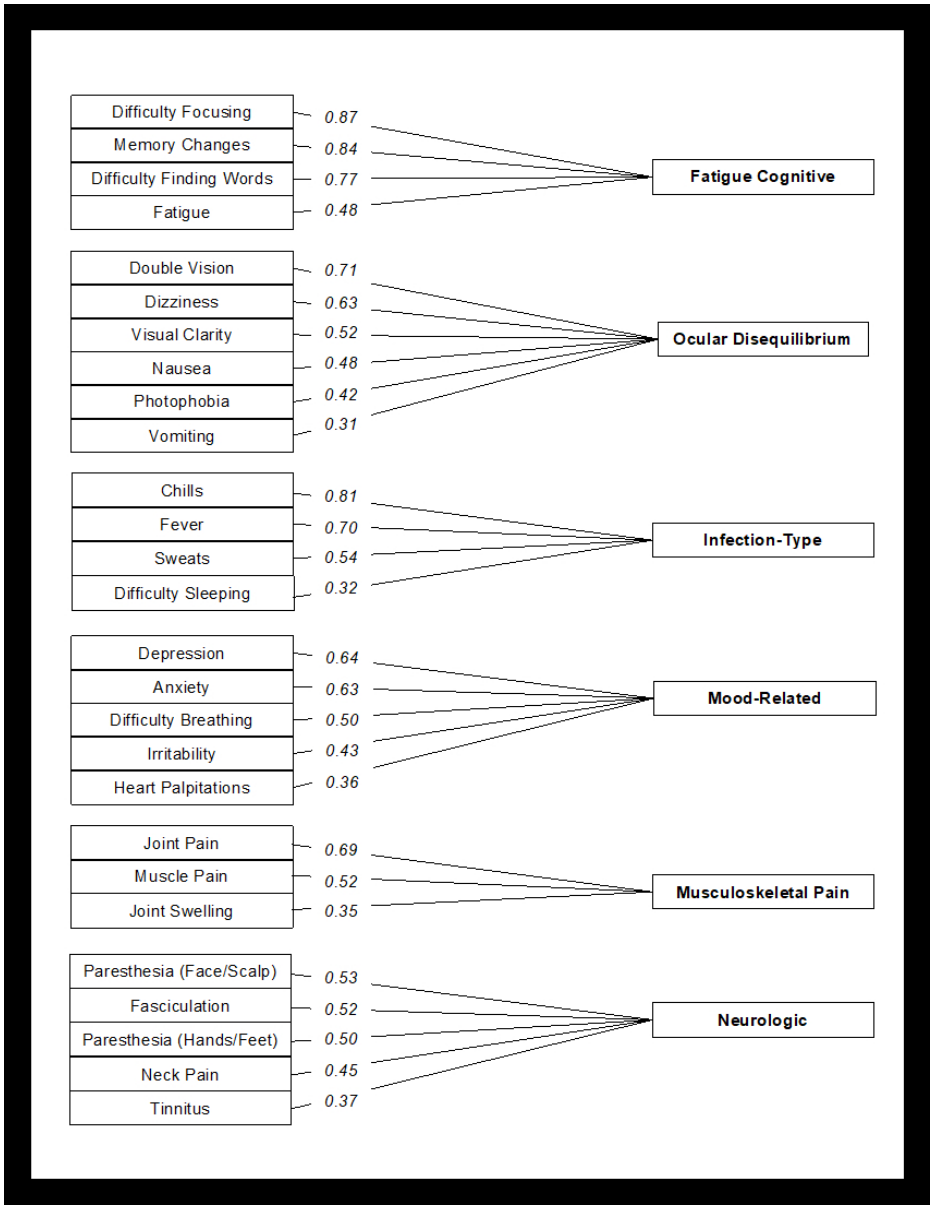
37 Fallon BA, Nields JA. Lyme disease: a neuropsychiatric illness. *Am J Psychiatry* 1994;**151**:1571–83.http://www.ncbi.nlm.nih.gov/pubmed/7943444

38 McLean CP, Asnaani A, Litz BT, *et al.* Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res* 2011;**45**:1027–35. doi:10.1016/j.jpsychires.2011.03.006

39 Albert PR. Why is depression more prevalent in women? J. Psychiatry Neurosci. 2015;**40**:219–21. doi:10.1503/jpn.150205

40 Tennant C. Life events, stress and depression: a review of recent findings. *Aust N Z J Psychiatry* 2002;**36**:173–82. doi:10.1046/j.1440-1614.2002.01007.x

41 Broadbent E, Petrie KJ. Symptom perception. In: Ayers S, Baum A, McManus C, *et al.*, eds. *Cambridge Handbook of Psychology, Health and Medicine, Second Edition*. Cambridge University Press 2007. 219–23. doi:10.1017/CBO9780511543579.048



Exploratory factor analysis of 30 common PTLD symptoms suggests a 6-factor model. Three of the symptoms did not load and were dropped in the final model.

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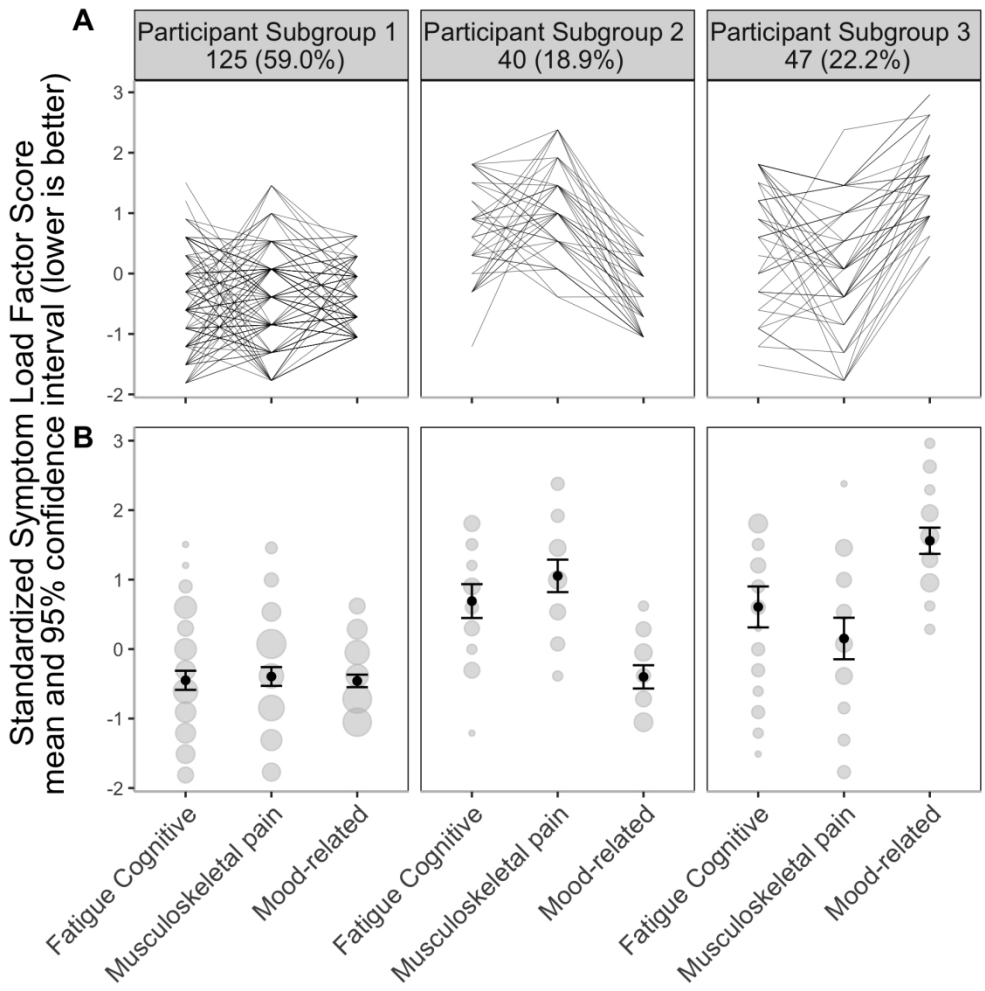
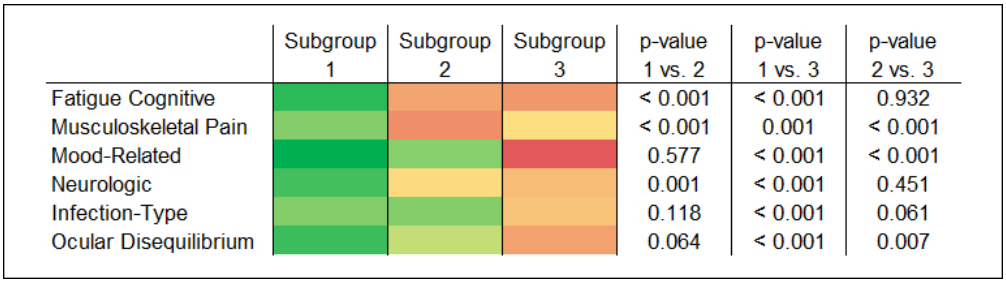


Three subgroups of participants identified based on latent profile analysis (panels A and B).

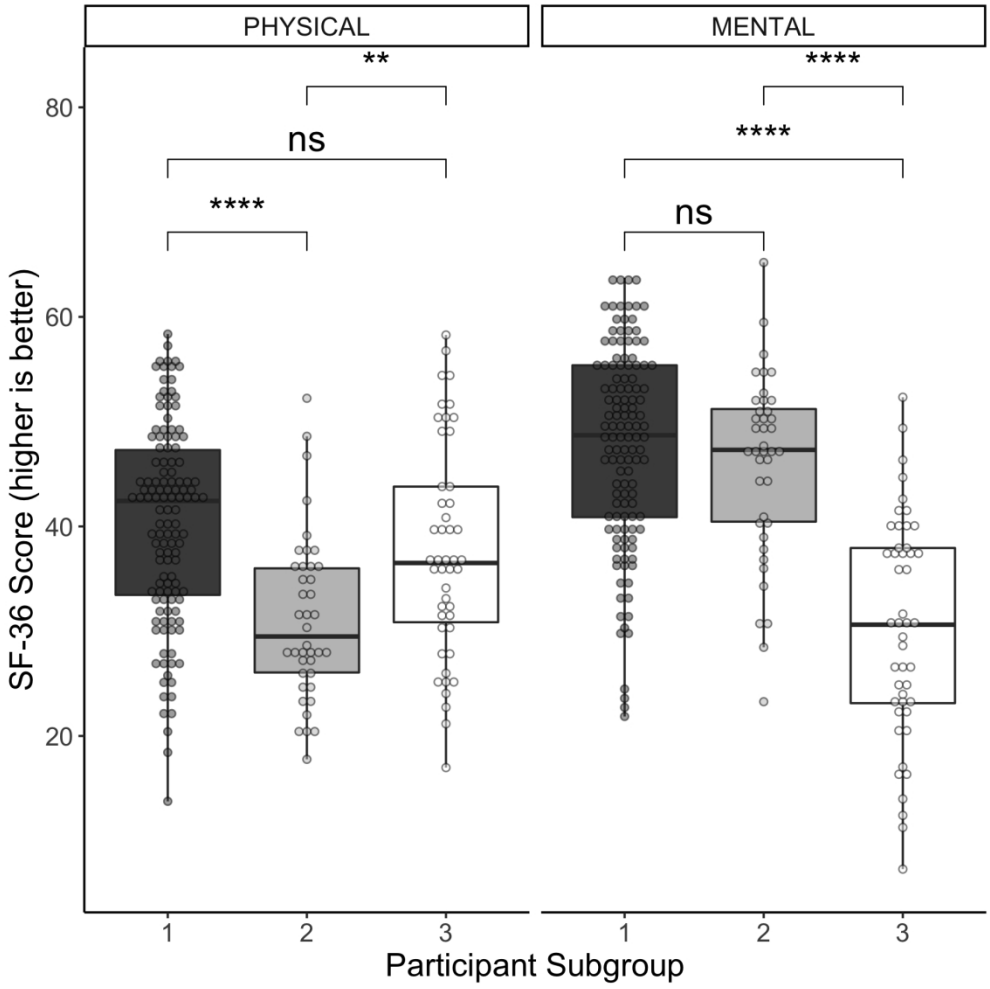
165x165mm (300 x 300 DPI)



	Subgroup 1	Subgroup 2	Subgroup 3	p-value 1 vs. 2	p-value 1 vs. 3	p-value 2 vs. 3
Fatigue Cognitive				< 0.001	< 0.001	0.932
Musculoskeletal Pain				< 0.001	0.001	< 0.001
Mood-Related				0.577	< 0.001	< 0.001
Neurologic				0.001	< 0.001	0.451
Infection-Type				0.118	< 0.001	0.061
Ocular Disequilibrium				0.064	< 0.001	0.007

Participant subgroup differences in median standardized symptom factor-based scores

65x17mm (300 x 300 DPI)



SF-36 health-related quality of life physical and mental component scores[18] for the three patient subgroups. ns = Not Significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

165x165mm (300 x 300 DPI)

SUPPLEMENTARY MATERIAL

Table S1. Exploratory Factor Analysis Loading Matrix

	Fatigue Cognitive	**Ocular Disequilibrium**	**Infection -type**	**Mood-related**	**Musculoskeletal pain**	**Neurologic**	*Max loading*	*Second largest loading*	*Difference between max and second largest loading*
Fever	0.05	-0.07	0.70	-0.17	0.02	-0.06	*0.70*	*0.05*	*0.65*
Chills	-0.07	0.06	0.81	0.08	-0.04	0.07	*0.81*	*0.08*	*0.73*
Sweats	0.07	0.06	0.54	0.01	0.15	-0.11	*0.54*	*0.15*	*0.39*
Fatigue	0.48	0.00	0.12	0.15	0.25	-0.04	*0.48*	*0.25*	*0.23*
Muscle Pain	0.13	-0.02	0.09	0.09	0.52	0.20	*0.52*	*0.2*	*0.32*
Joint Pain	0.11	0.00	0.06	-0.09	0.69	0.14	*0.69*	*0.14*	*0.55*
Joint Swelling	-0.07	0.14	0.06	-0.13	0.35	0.05	*0.35*	*0.14*	*0.21*
Numbness hands/feet	-0.02	0.18	0.00	-0.10	0.14	0.50	*0.50*	*0.18*	*0.32*
Numbness face	-0.06	0.21	-0.01	-0.07	0.02	0.53	*0.53*	*0.21*	*0.32*
Muscle twitching	0.01	-0.02	0.05	0.11	0.19	0.52	*0.52*	*0.19*	*0.33*
Headache	0.22	0.21	0.11	0.14	-0.02	-0.01	*0.22*	*0.21*	*0.01*
Eyes sensitive to light	0.05	0.42	0.12	0.15	0.06	0.16	*0.42*	*0.16*	*0.26*
Changes in vision clarity	-0.02	0.52	0.16	0.10	-0.04	0.14	*0.52*	*0.16*	*0.36*
Double vision	0.03	0.71	-0.01	-0.15	-0.06	0.03	*0.71*	*0.03*	*0.68*
Dizziness	0.09	0.63	0.01	0.12	0.05	0.05	*0.63*	*0.12*	*0.51*
Ringing in ears	0.01	0.01	0.15	0.12	-0.06	0.37	*0.37*	*0.15*	*0.22*
Neck pain	0.22	-0.10	-0.01	0.06	0.13	0.45	*0.45*	*0.22*	*0.23*
Low back pain	0.24	-0.01	0.04	0.05	0.33	0.13	*0.33*	*0.24*	*0.09*
Poor coordination	0.32	0.32	-0.03	0.07	0.32	-0.06	*0.32*	*0.32*	*0.00*
Memory changes	0.84	0.05	0.04	-0.04	0.02	-0.06	*0.84*	*0.05*	*0.79*
Difficulty finding words	0.77	0.03	-0.05	0.08	0.05	0.01	*0.77*	*0.08*	*0.69*
Difficulty focusing	0.87	0.00	0.00	0.01	-0.03	0.07	*0.87*	*0.07*	*0.80*
Heart palpitations	-0.03	0.24	-0.03	0.36	0.25	-0.09	*0.36*	*0.25*	*0.11*

Difficulty breathing	-0.17	0.08	0.12	0.50	0.35	0.00	*0.50*	*0.35*	*0.15*
Nausea	0.03	0.48	0.10	0.20	0.04	-0.07	*0.48*	*0.2*	*0.28*
Vomiting	0.15	0.31	0.16	-0.07	-0.07	-0.17	*0.31*	*0.16*	*0.15*
Difficulty sleeping	0.10	0.02	0.32	0.17	0.21	0.06	*0.32*	*0.21*	*0.11*
Anxiety	0.19	0.10	0.04	0.63	-0.10	0.06	*0.63*	*0.19*	*0.44*
Depression	0.10	0.00	-0.01	0.64	-0.02	0.02	*0.64*	*0.1*	*0.54*
Irritability	0.27	-0.01	0.14	0.43	-0.29	0.22	*0.43*	*0.27*	*0.16*

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	4-5, 7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, included in footnotes for Tables 1 and 2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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TITLE

Symptom Heterogeneity and Patient Subgroup Classification among US Patients with Post-Treatment Lyme Disease: An Observational Study

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ABSTRACT

Objectives: To identify underlying subgroups with distinct symptom profiles, and to characterize and compare these subgroups across a range of demographic, clinical, and psychosocial factors, within a heterogeneous group of patients with well-defined post-treatment Lyme disease.

Design: A clinical case series of patents.

Setting: Participants were recruited from a single-site, Lyme disease referral clinic patient population and were evaluated by physical exam, clinical laboratory testing, and standardized questionnaires.

Participants: Two hundred and twelve participants met study criteria for post-treatment Lyme disease, with medical record-confirmed prior Lyme disease as well as current symptoms and functional impact.

Results: Exploratory factor analysis classified 30 self-reported symptoms into six factors: "Fatigue Cognitive," "Ocular Disequilibrium," "Infection-Type," "Mood-Related," "Musculoskeletal Pain," and "Neurologic." A final latent profile analysis was conducted using "Fatigue Cognitive", "Musculoskeletal Pain", and "Mood-Related" factor-based scores, which produced three emergent symptom profiles, and participants were classified into corresponding subgroups with 59.0%, 18.9%, and 22.2% of the sample, respectively. Compared to the other two groups, subgroup 1 had similarly low levels across all factors relative to the sample as a whole, and reported lower rates of disability (1.6% vs. 10.0%, 12.8%; q=0.126, 0.035) and higher self-efficacy (median: 7.5 vs. 6.0, 5.3; q=0.068, <0.001). Subgroup 2 had the highest "Musculoskeletal Pain" factor-based scores (q ≤0.007). Subgroup 3 was characterized overall by higher symptom factor-based scores, and reported higher depression (q ≤0.001).

Conclusions: This analysis identified six symptom factors and three potentially clinically relevant subgroups among patients with well-characterized post-treatment Lyme disease. We found that these subgroups were differentiated not only by symptom phenotype, but also by a range of other factors. This may serve as an initial step towards engaging with the symptom heterogeneity that has long been observed among patients with this condition.

Keywords: Lyme disease, post-treatment Lyme disease, symptoms, patient subgroups

ARTICLE SUMMARY

Strengths and limitations of this study

- We operationalized a rigorous definition of post-treatment Lyme disease in our sample population, which ensured greater specificity of our findings to patients whose current illness is more evidently linked to prior Lyme disease.
- This specificity, and the regional focus of our sample population, may limit generalizability to the larger population of patients with persistent symptoms following treatment for Lyme disease, or those from other regions of the US.
- Reproducibility of the subgroup analysis may be affected by necessary methodological decisions incorporating statistical and clinical criteria which were made during the analytic process.
- We were able to draw upon a relatively large sample size of participants with well-characterized post-treatment Lyme disease, which allowed for clear and concise interpretability of data.

INTRODUCTION

Lyme disease is a tick-borne disease of increasing public health importance found primarily across temperate regions of the Northern Hemisphere.[1,2] Clinical signs of early infection may include a round, red, skin lesion occurring at the site of the bite of infected *Ixodes* ticks, and/or a transient, non-specific illness consisting of fever, fatigue, myalgia, or arthralgia.[1,3] If not promptly identified or otherwise left untreated, the bacteria (*Borrelia burgdorferi* in the United States) can disseminate to other areas of the skin, and via the blood stream to other organs such as the nervous system, heart, and joints.[4] Consequently, although less commonly observed, patients with untreated infection can present with objective, later manifestations of neurologic disease, carditis, or arthritis.[3]

While the majority of patients treated appropriately for Lyme disease recover, a subset develop a poorly-understood, chronic illness of persistent or recurrent symptoms following treatment.[5] The presence of chronic or persistent symptoms following acute infection has been documented in a subset of patients for a number of viral and bacterial pathogens.[6] Although more research is needed, the symptom phenotype of these illnesses, including that of the newly described “long COVID” shares many overlapping characteristics.[6,7] In order to methodically

advance scientific understanding, a standardized, highly-specific, research definition for post-treatment Lyme disease syndrome (PTLD, alternatively previously called post-Lyme disease syndrome or post-treatment Lyme disease) has been used and operationalized to identify a subset of these patients with on-going symptoms linked temporally to strong evidence of prior exposure to *B. burgdorferi*.[8–10] The most prominent symptoms, and those included in the Infectious Diseases Society of America's (IDSA) proposed case definition of PTLD,[3] include fatigue, musculoskeletal pain, and cognitive dysfunction. However, patients with PTLD often also report a broad range of other neurologic, sleep, mood, ocular, and other symptoms.[9,11,12] This heterogeneity is often compounded by the significant impact of these symptoms on patient quality of life and functioning.[9,13] Additionally, given the lack of: a) a sensitive and specific test to aid diagnosis, b) FDA-approved treatment options for patients, and c) a known etiology, PTLD presents a complex challenge to physicians.

As large studies among patients with well-characterized PTLD have not been conducted, this diversity in PTLD symptom reporting has not been comprehensively examined and it is unknown whether it may obscure the presence of distinct clinical patient subgroups. However, it is increasingly common that through advances in personalized medicine, diseases previously considered a single entity have been found instead to be comprised of clinically and/or biologically coherent subgroups.[14,15] Furthermore, similar to fibromyalgia, PTLD is likely a complex, multifactorial illness with immunologic, microbiologic, genetic, and/or psychosocial factors contributing to disease development, severity, and persistence.[5,16] Consequently, examining the heterogeneity of clinical presentations and symptom reporting that exists among patients with PTLD is important because it may inform a deeper understanding of etiology and effective treatment approaches. Therefore, the aims of this observational study were a) to identify underlying patient subgroups with distinct symptom profiles within a heterogeneous group of patients with well-defined PTLD, and b) to characterize and compare these subgroups across a range of demographic, clinical, laboratory, and psychosocial factors.

METHODS

Study Participants

Participants were recruited from a referral-based clinic population. Detailed recruitment information and enrollment criteria for this study were included in an initial publication

describing a subset of the larger sample of participants included in the current analysis.[9] In brief, we replicated much of the criteria set forth in the IDSA's proposed case definition for PTLD through our eligibility criteria.[3,9] Participants were required to have prior evidence in their medical record of appropriately treated, CDC-definite or probable Lyme disease.[17] They were also required to have current, functionally-impairing fatigue, pain, and/or cognitive dysfunction, and were excluded for a range of specific co-morbid medical conditions, as previously described.[9] For the current analysis, we did not limit the sample to those with greater than six month's illness duration, and thus, we refer to our sample as meeting criteria for post-treatment Lyme disease (PTLD). The Institutional Review Board of the Johns Hopkins University School of Medicine approved this study, and written informed consent was obtained from all study participants.

Patient and Public Involvement

Patients and the public were not directly involved in the design, recruitment, or assessment of this study.

Data Collection Instruments

Participants were asked to self-administer a 36-item symptom questionnaire (PLQS) developed based on prior clinical and research experience among patients with PTLD.[9] Participants indicated both presence and severity over the past two weeks for each symptom (0=absent, 1=mild, 2=moderate, or 3=severe). Of the original 36 symptoms, we excluded the following, which occurred with low frequency in our sample and were not considered to be core symptoms of PTLD (the percent endorsed at a moderate or severe level): urination pattern change (9%), diarrhea (9%), sore throat (4%), drooping eyelid(s) (2%), Bell's palsy (1%), and tender lymph nodes (2%). Data from the remaining 30 symptoms provided the basis for the subgroup analyses described below (see Supplemental Table S1 for the complete list of symptoms).

Participants were also asked to self-administer a battery of additional questionnaires included in the current analyses. The Beck Depression Inventory-II is a 21-item depression metric which can be divided into 'Somatic' and 'Cognitive-Affective' subscales.[18,19] In order to avoid duplication with other variables in this analysis, only the 'Cognitive-Affective' subscale (BDI-C/A) was included, which has a total score of 0-48. Quality of life was measured by the

Short-Form Health Survey, Version 2 (SF-36).[20] This 36-item metric can be summarized into Physical and Mental Component Scores (PCS and MCS, respectively), with a higher score indicating higher quality of life. These scores can also be compared with the US population mean (50.0 ± 10.0). The Life Events Checklist (LEC) is a 17-item measure with total scores of 0-17 of prior potentially traumatic events originally developed to aid in the diagnosis of post-traumatic stress disorder.[21] The Stanford Chronic Disease Self-Efficacy Scale (CDSE) is a 6-item measure of perceived self-efficacy for chronic disease self-management.[22,23] The Big Five Inventory (BFI) is a 44-item measure of five personality dimensions; extraversion, agreeableness, conscientiousness, emotional stability, and openness.[24–26] Variables related to prior, initial Lyme disease clinical presentation, treatment(s), and duration of illness were abstracted from participants' medical records from the time of Lyme disease onset. Participants self-reported other prior medical diagnoses as part of a structured clinical interview.

During the study visit, a physical exam was performed which included routine measures of height, weight, pulse, and blood pressure. Body mass index (BMI) was calculated using the standard formula (weight [kg] / height [m^2]). Vibratory index was measured on the distal interphalangeal joint of the index finger and on the interphalangeal joint of the hallux using a Rydel-Seiffer 64 Hz tuning fork.[27] Lastly, participants underwent a blood draw, and standard clinical tests (CBC, CMP, C-reactive protein, and two-tier serology for antibodies to *B. burgdorferi*) were performed by a large, commercial laboratory.

Statistical Analysis

We hypothesized that sub-collections of symptoms are caused by different but interrelated underlying biological mechanisms, which are not directly observable in our study. Therefore, we first performed exploratory factor analysis (EFA) to identify the latent relational structure of the symptoms included in the PLQS, which subsequently also reduced the dimensionality of the data. The Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) and Bartlett's test of sphericity were used to check whether the data were suitable for factor analysis. Considering the ordinal nature of the variables, both polychoric and Pearson's correlation coefficients were used. We chose the minimal residual estimation method because it can be used when the sample size is relatively small and when the correlation matrix is non-positive definite.[28] Oblique rotation was used to allow for correlations between extracted

factors. The number of retained factors was informed by the visual scree test and parallel analysis, while taking into consideration clinical meaningfulness and the balance between parsimony and comprehensiveness. We used a factor loading cutoff value of 0.3.

Next, to uncover subgroups of participants we performed latent profile analysis (LPA) on the standardized symptom factor-based scores generated by the EFA. The number of identified clusters was determined based on minimization of the Bayesian information criteria (BIC) and the correlational structure of the data. Lastly, pairwise sub-group differences were examined and summarized using 2-sample t test or Wilcoxon rank sum test for continuous variables and chi-squared or Fisher's exact test for categorical variables. Considering the accumulation of type 1 error across multiple hypothesis tests, we calculated q values to control false discovery rate (FDR) at 5%.[29] All statistical analyses were performed using R (version 3.6.1).

RESULTS

Participant Characteristics

A total of 225 participants with PTLD were enrolled in the study. We excluded six participants whose PTLD symptoms began more than six months after their initial Lyme disease episode, and seven participants who missed all symptom variables on the PLQS, for a total of 212 in the final sample. We employed mean imputation for three participants who each missed one of the 30 PLQS variables included in the analysis. Table 1 shows a description of the final participant sample. The average age was 48 years and there was a slight (58.5%) majority male in the sample. A large majority were residents of Mid-Atlantic states at the time of their disease onset (93.4%) and/or residents of states considered 'high-incidence' for Lyme disease (96.7%).[30]

Table 1. Characteristics of 212 participants with well-defined post-treatment Lyme disease[a]

	All Participants n = 212
Age at study visit	48.00 [37.00, 58.00] (18.00, 82.00)
Male gender	124 (58.5%)
White, non-Hispanic	190 (89.6%)
Years of education	16.00 [14.00, 18.00] (10.00, 30.00)

Annual household income >$100K	119/203 (58.6%)
Currently out of work on disability	12 (5.7%)
Lyme disease onset while resident of CDC Lyme disease 'high-incidence' state[30]	205 (96.7%)
CDC 'confirmed' initial Lyme disease presentation[17]	124 (58.5%)
Duration of illness from onset of PTLD symptoms to study visit (years)	1.67 [0.68, 3.81] (0.06, 28.59)
Total antibiotic exposure from symptom onset (weeks)	8.57 [4.43, 14.29] (2.00, 168.57)

[a]Data from categorical variables are presented as count (%). Data from normally distribute variables are presented as mean ± standard deviation (range) and from continuous variables without normal distribution as median [25th percentile, 75th percentile] (range). Proportions were calculated based on non-missing data and may not add to 100% because of rounding. Missing data are as follows: Years of education, 1 (0.5%); Annual household income, 9 (4.2%).

Latent Relational Structure among Symptoms

The total symptom score among patients with PTLD ranged from 2 to 70, with a median and first and third quartile interval of 22 (14, 33). Histograms of individual symptom scores are presented in Supplemental Figure S1. In the EFA analysis, the original polychoric correlation matrix was non-positive definite. After smoothing was performed to arrive at a positive definite matrix, it resulted in a poor overall sampling adequacy index (0.10) and an ultra-Heywood case was detected. However, the overall measure of sampling adequacy based on the Pearson's correlation coefficient was 0.86 (meritorious), and Bartlett's test of sphericity was significant ($p<0.001$). A 6-factor model was suggested by both statistical criteria and clinical meaningfulness (Figure 1, see Supplemental Table S1 for the complete factor pattern matrix). The root mean square of the residuals was 0.04, the root mean square error of approximation index was 0.06, and the Tucker Lewis index of factoring reliability was 0.85. The symptom headache did not significantly load to any factor (maximum loading: 0.22, Supplemental Table S1). Poor coordination and lower back pain loaded weakly to multiple factors (maximum loading ≤ 0.33), and had close cross loading (difference less than 0.10) across two or more factors, and were therefore removed. The percent endorsed at a moderate or severe level for these symptoms was 15.6%, 4.2%, and 9.4%, respectively. An expert physician on the study team (JA) named the factors as "Fatigue Cognitive," "Ocular Disequilibrium," "Infection-Type," "Mood-Related," "Musculoskeletal

Pain," and “Neurologic.” All six factors were weakly or moderately correlated with each other (0.21 to 0.41), with the strongest correlation between the “Fatigue Cognitive” and “Mood-Related” factors. For a more straightforward interpretation, six factor-based scores were calculated for each participant by adding up the scores of the symptoms within each factor, and then these factor-based scores were standardized to have a mean of zero and a standard deviation of one.

Participant Subgroup Analysis

For the LPA analysis, we did not include the “Ocular Disequilibrium” factor as it prevented the LPA from converging for most of the specified models in model selection, possibly due to its low endorsement rate (the percentage endorsing symptoms included in this factor at a moderate or severe level ranged from 0.9% to 24.1%). When conducted on the remaining five factors, LPA classified participants into two groups based on their overall level of symptom reporting (high vs. low) relative to the sample as a whole.

We then conducted a secondary LPA incorporating those factors which contained only the most common PTLD-defining symptoms as well as mood (i.e. “Fatigue Cognitive”, “Musculoskeletal Pain”, and “Mood-Related”). Three symptom profiles emerged (Figure 2) and participants were classified into subgroups corresponding to these symptom profiles. Subgroup 1 contained 59.0% of the participants and was characterized by similarly low levels across all three factors relative to the sample as a whole. Subgroups 2 and 3 contained 18.9% and 22.2% of the participants, respectively, and were characterized by overall higher levels of the three factors relative to the entire sample. These results remained stable when the “Neurologic” factor was re-introduced in the LPA.

Participant Subgroup Comparisons

We first compared the three subgroups generated by the LPA across all six original PLQS factor-based symptom scores (Figure 3). Compared to subgroup 1, “Fatigue Cognitive” and “Neurologic” factor-based scores were significantly higher among both subgroup 2 and 3 participants. “Musculoskeletal Pain” was the only factor to statistically significantly differentiate all three subgroups from one another, with scores in subgroup 1 being the lowest and subgroup 2 being the highest. “Infection-Type” and “Ocular Disequilibrium” factor scores trended in the

direction of increasing from subgroup 1 to 3. Lastly, "Mood-Related" factor scores were significantly higher among subgroup 3 participants compared both to subgroups 1 and 2, which did not differ significantly from each other.

Results of detailed demographic, clinical, laboratory, and psychosocial characteristic comparisons by subgroup are presented in Table 2. Notably, neither the percentage male ($p \geq 0.887$ for all pair-wise comparisons) nor LEC total score ($p \geq 0.615$ for all pair-wise comparisons) were statistically significantly different across subgroups. Participants in subgroup 1, which generally included those with lower symptom factor-based scores, also reported lower rates of being on disability than the other two groups and had higher CDSE scores. Subgroup 2 was found to have higher blood pressure, and a higher percentage of participants with an abnormal C-reactive protein than subgroup 1.

Overall, participants in subgroup 3 were younger, with a lower percentage reporting an annual household income > $100,000. This group was also found to have a median illness duration of almost a year longer than the other two groups, and a higher percentage who reported prior IV antibiotic treatment. Consistent with the pattern of symptom reporting in the factor-based PLQS scores, subgroup 3 had significantly worse BDI-C/A scores than the other two subgroups. On the BFI, subgroup 3 had significantly lower scores in the Conscientiousness and Emotional Stability domains than the other two subgroups.

Those co-morbid diagnoses occurring with at least 5% prevalence in the sample as a whole are also reported in Table 2. No statistically significant differences were found for any of the conditions with the exception that participants in subgroup 3 were almost three times as likely as those in subgroup 1 to report migraine headaches. In examining differences by subgroup in SF-36 quality of life scores, we found that subgroup 2 had significantly lower PCS scores compared to the other two groups, whereas subgroup 3 had significant lower MCS scores compared to the other two groups (Figure 4). This is consistent with the pattern of symptom reporting in the factor-based scores which differentiated the three groups.

Table 2. Patient subgroup comparisons across demographic, clinical laboratory, and psychosocial characteristics[a]

	Subgroup 1 *n*=125	Subgroup 2 *n*=40	Subgroup 3 *n*=47	*q*-value 1 vs. 2	*q*-value 1 vs. 3	*q*-value 2 vs. 3
DEMOGRAPHIC						
Age at study visit (years)	49.00 [40.00, 61.00] (18.00, 82.00)	51.00 [40.75, 56.00] (25.00, 70.00)	42.00 [27.00, 52.00] (18.00, 82.00)	0.887	0.126	0.219
Male gender	75 (60.0%)	23 (57.5%)	26 (55.3%)	1.000	0.887	1.000
White, non-Hispanic	111 (88.8%)	34 (85.0%)	45 (95.7%)	0.887	0.528	0.370
Years of education	16.00 [14.00, 18.00] (10.00, 25.00)	16.00 [14.00, 18.00] (12.00, 30.00)	16.00 [14.25, 18.00] (12.00, 22.00)	0.937	0.887	0.859
Annual household income >$100K	78/117 (66.7%)	23 (57.5%)	18/46 (39.1%)	0.697	0.014	0.370
Out of work on disability	2 (1.6%)	4 (10.0%)	6 (12.8%)	0.126	0.035	0.887
Body mass index (kg/m^2)	25.72 [22.71, 29.42] (16.47, 38.88)	26.78 [22.59, 30.50] (19.80, 41.74)	26.15 [23.47, 29.29] (18.99, 45.70)	0.615	0.849	0.887
CLINICAL/PHYSICAL EXAM						
Illness duration from disease onset to study visit (years)	1.45 [0.59, 3.84] (0.15, 28.59)	1.30 [0.71, 2.14] (0.06, 13.13)	2.23 [1.03, 5.56] (0.13, 18.67)	0.720	0.309	0.086
CDC 'confirmed' initial Lyme disease[17]	77 (61.6%)	21 (52.5%)	26 (55.3%)	0.707	0.849	1.000
Initial late Lyme arthritis	15 (12.0%)	3 (7.5%)	1 (2.1%)	0.849	0.249	0.615
Initial neurologic Lyme disease	7 (5.6%)	2 (5.0%)	7 (14.9%)	1.000	0.299	0.404
Time to initial recommended antibiotic treatment (days)[b]	23.00 [0.00, 110.00] (0.00, 10000.00)	14.50 [0.00, 181.25] (0.00, 757.00)	14.00 [2.50, 128.00] (0.00, 3700.00)	0.887	0.887	0.715
Total antibiotic exposure since disease onset (weeks)	8.00 [4.43, 13.00] (2.00, 112.86)	7.64 [4.29, 19.21] (3.00, 130.00)	9.00 [5.64, 14.71] (2.86, 168.57)	0.923	0.566	0.863
Intravenous antibiotic use	26 (20.8%)	7 (17.5%)	20 (42.6%)	0.923	0.035	0.092
Non-recommended antibiotic exposure prior to recommended antibiotic exposure[b]	17 (13.6%)	4 (10.0%)	8 (17.0%)	0.909	0.887	0.831
Steroid exposure after disease onset, prior to recommended antibiotic treatment[b]	10 (8.0%)	7 (17.5%)	4 (8.5%)	0.391	1.000	0.615
Systolic blood pressure (mmHg)	125.50 [114.00, 137.50] (92.00, 171.00)	133.00 [121.75, 144.25] (106.00, 173.00)	126.00 [115.00, 138.00] (99.00, 179.00)	0.091	0.937	0.288
Diastolic blood pressure (mmHg)	80.82 ± 9.36 (63.00, 103.00)	85.53 ± 9.34 (64.00, 110.00)	82.47 ± 8.93 (63.00, 100.00)	0.035	0.597	0.358

Pulse (beats per minute)	68.00 [61.50, 73.00] (48.00, 120.00)	70.50 [64.00, 81.00] (52.00, 106.00)	70.00 [64.00, 80.25] (51.00, 104.00)	0.199	0.402	0.859
Vibratory sense abnormal[c]	34/124 (27.4%)	15/39 (38.5%)	10/45 (22.2%)	0.566	0.882	0.402
CO-MORBIDITIES						
Thyroid disease	9 (7.2%)	4 (10.0%)	4 (8.5%)	0.816	0.887	1.000
Heart disease or Hypertension	20 (16.0%)	5 (12.5%)	7 (14.9%)	0.916	1.000	1.000
Migraine headaches	17 (13.6%)	10 (25.0%)	18 (38.3%)	0.386	0.007	0.566
Carpal tunnel syndrome	13 (10.4%)	5 (12.5%)	4 (8.5%)	0.901	1.000	0.887
Neuropathy/neuromuscular disorder	8 (6.4%)	3 (7.5%)	6 (12.8%)	0.887	0.597	0.797
LABORATORY						
Absolute lymphocyte count ($10^3/\mu L$)	1.96 [1.56, 2.19] (0.68, 3.82)	1.89 [1.59, 2.26] (1.09, 4.29)	1.87 [1.63, 2.29] (0.82, 3.26)	1.000	0.711	0.887
C-reactive protein abnormal	6/119 (5.0%)	8/38 (21.1%)	3/43 (7.0%)	0.035	0.887	0.309
Reactive IgG bands on two-tier testing for antibodies to *B. burgdorferi*	5.00 [2.00, 8.00] (0.00, 10.00)	4.00 [2.00, 7.00] (0.00, 10.00)	4.00 [2.00, 6.50] (0.00, 10.00)	0.720	0.391	0.887
PSYCHOSOCIAL						
Beck Depression Inventory-II Cognitive/Affective subscale score[19]	5.00 [1.00, 8.00] (0.00, 20.00)	6.00 [4.00, 8.00] (0.00, 17.00)	13.00 [9.00, 19.00] (3.00, 39.00)	0.528	< 0.001	< 0.001
Stanford Chronic Diseases Self-Efficacy total score[22,23]	7.50 [5.30, 8.50] (1.00, 9.80)	6.00 [4.30, 7.55] (1.00, 9.80)	5.30 [4.25, 6.80] (1.00, 9.70)	0.068	< 0.001	0.597
Life Events Checklist total score[21]	2.00 [1.00, 4.00] (0.00, 13.00)	2.00 [0.00, 3.25] (0.00, 8.00)	2.00 [0.50, 4.00] (0.00, 9.00)	0.615	0.879	0.887
Big Five Inventory: Extraversion score[25]	3.38 [2.75, 3.88] (1.38, 5.00)	3.44 [3.00, 3.91] (1.63, 4.88)	3.13 [2.56, 3.63] (1.75, 5.00)	0.797	0.527	0.355
Big Five Inventory: Agreeableness score	4.00 [3.67, 4.44] (2.44, 5.00)	4.22 [3.97, 4.56] (2.33, 5.00)	3.89 [3.38, 4.38] (2.33, 5.00)	0.299	0.408	0.084
Big Five Inventory: Conscientiousness score	4.00 [3.56, 4.44] (2.22, 5.00)	4.05 [3.67, 4.44] (2.22, 4.89)	3.67 [3.28, 4.11] (1.56, 4.89)	0.887	0.020	0.035
Big Five Inventory: Emotional Stability score	3.63 [3.13, 4.10] (1.38, 5.00)	3.75 [3.22, 4.25] (2.50, 5.00)	2.63 [1.82, 3.25] (1.00, 4.63)	0.668	< 0.001	< 0.001
Big Five Inventory: Openness score	3.70 [3.30, 4.20] (2.30, 5.00)	3.90 [3.40, 4.32] (2.70, 4.90)	3.80 [3.30, 4.10] (1.20, 4.80)	0.495	1.000	0.615

[a]Data from categorical variables are presented as count (%). Data from normally distribute variables are presented as mean ± standard deviation (range) and from continuous variables without normal distribution as median [25th percentile, 75th percentile] (range). Proportions were calculated based on non-missing data and may not add to 100% because of rounding. Missing data are as follows:

years of education, 1 (0.5%); annual household income, 9 (4.2%); body mass index, 18 (8.5%); systolic blood pressure, 5 (2.4%); diastolic blood pressure, 4 (1.9%); pulse, 3 (1.4%); vibratory sense, 4 (1.9%); absolute lymphocyte count, 2 (0.9%); C-reactive protein, 12 (5.7%); IgG reactive bands, 1 (0.5%); Beck Depression Inventory-II Cognitive/Affective score, 1 (0.5%); Stanford Chronic Diseases Self-Efficacy score, 1 (0.5%); Big Five Inventory, 3 (1.4%). [b]Recommended antibiotic regimens were considered any of the following: Doxycycline 100mg BID for ≥ 10 days, Tetracycline 500mg TID for ≥ 14 days, Ceftin 500mg BID for ≥ 14 days, Ceftriaxone 2g Q24 ≥ 14 days. Other drugs, or lower doses or durations were considered non-recommended antibiotic regimes. [c]Below age-adjusted normal vibration threshold values in either upper (distal interphalangeal joint of the index finger) or lower (interphalangeal joint of the hallux) extremities on either right or left side using a Rydel-Seiffer 64 Hz tuning fork.[27]

DISCUSSION

PTLD is a complex illness which is characterized by a wide range of clinical symptoms that can significantly impact quality of life for many patients.[9,11–13] The aim of this study was to examine heterogeneity in symptom reporting in order to ultimately identify and characterize clinically relevant patient subgroups. Using our PLQS questionnaire, we first identified six symptom-based factors through EFA analysis. The relational structure of these results had overall clinical face validity, with symptoms clustering in seemingly physiologically relevant rather than randomly distributed ways. For example, all three cognitive symptoms loaded onto the same factor, as did joint pain, muscle pain, and joint swelling. Furthermore, the six factors we identified represent commonly recognized domains in the clinical phenotype of PTLD.

Although the analyses and the measure differed, results from our EFA were generally consistent with those from a recent study with some participant sample overlap, which aimed to validate the General Symptom Questionnaire-30 (GSQ-30) in PTLD.[31] One noticeable difference was that fatigue loaded with the musculoskeletal pain factor in the GSQ-30 study rather than with cognitive symptoms, as it did in the current study. This suggests that fatigue in PTLD could arise from multiple sources including pain, the central nervous system, or muscle weakness. Similarly, insomnia may also be a multifactorial symptom, as it showed low loading (0.32) to the 'Infection-Type' factor in the current study, with significant cross-loading to the 'Fatigue Cognitive', 'Musculoskeletal Pain', and 'Mood-Related' factors.

Several additional symptom factor loadings were informative as well. Neck pain is relatively common in the general population,[32] however it is reported with greater frequency and severity in this sample population compared to controls,[9] and the cause is unknown. Given that neck pain loaded the strongest onto the 'Neurologic' factor, with the second strongest loading to 'Fatigue Cognitive' and not 'Musculoskeletal Pain', we hypothesize the potential for a neurologic rather than arthritic origin. We also found that difficulty breathing and heart palpitations loaded onto the 'Mood-Related' factor, implying that this constellation of symptoms may result from a common pathway such as autonomic nervous system activation or central sensitization[33] rather than specific cardiac or pulmonary pathology. Alternatively, anxiety and other mood-related symptoms could result secondary to experiencing these types of distressing physiologic symptoms. The hypothetical relational constructs we uncovered using EFA may

shed light on, but not necessarily equate to, distinct biological mechanisms resulting in symptoms. Some symptoms may have a composite underlying mechanism, some may correlate with each another despite different mechanisms, and some distinct factors could represent different sub-types of a shared general mechanism.

We then used a subset of the symptom-based factors in an LPA analysis to ultimately identify three patient subgroups corresponding to specific symptom profiles. This subgroup classification was prominently differentiated first by overall severity of symptom reporting, where high and low symptom reporters were identified. We plan to investigate factors associated with severity in the sample as a whole in future multivariate analyses. It is important to clarify that symptom severity in the current study is relative to this study sample of participants with PTLD and not the general population; we have previously shown a higher symptom burden in a subset of this sample of patients with PTLD compared to non-Lyme infected controls.[9]

Similar to our previous GSQ-30 study,[31] we conclude that morbidity in this population can exist above and beyond the effects of mood-related symptoms. Indeed, in our EFA analysis an independent "Mood-Related" factor was formed whose symptoms failed to load with other core symptoms of PTLD such as fatigue, pain, and cognitive difficulty. This is also supported by the pattern of symptom factor-based score reporting in subgroup 2. This subgroup had the highest "Musculoskeletal Pain" factor-based scores, however their 'Mood-Related" factor-based scores remained relatively low, similar to those of subgroup 1. This pattern also suggests that mood-related symptoms in PTLD may be more likely to be associated with fatigue or cognitive symptoms than with pain. Moreover, although fatigue/cognitive, mood-related, and pain symptoms all formed discrete factors in our analysis, "Mood-Related" factor scores were more strongly correlated with "Fatigue Cognitive" than they were with "Musculoskeletal Pain" scores (0.41 vs. 0.21, respectively).

We did define a subset of our sample (22.2%, subgroup 3) who overall reported significantly higher "Mood-Related" factor-based scores relative both to the other two subgroups and to their other symptom factor-based scores. Comparing subgroups across a variety of domains suggests several possible explanations for this finding. First, despite being younger, participants in subgroup 3 had a longer illness duration, as abstracted from their medical record. We would hypothesize that the effects of a chronic, often functionally impairing illness on mood would both compound over time and be more pronounced among younger patients. Second,

subgroup 3 also endorsed lower self-efficacy in managing their illness. This is unsurprising, as lower self-efficacy has been found to be associated with a higher degree of mood symptoms in a number of studies.[34,35] Furthermore, participants in subgroup 3 also scored lower on the Conscientiousness and Emotional Stability dimensions of the BFI, although additional research is warranted to explore the complex construct of personality among patients with PTLD. In sum, our findings suggest that participants in subgroup 3 may have been more psychologically vulnerable to the effects of a significant chronic illness over time when they first encountered Lyme disease. Indeed, many of the psychosocial variables that we measured have been shown to impact illness and resilience in other similar chronic disease populations.[36–38]

Finally, our data also suggest that participants with prior neurologic pathology may be over-represented in subgroup 3. Although the subgroup comparisons were not statistically significant, we observed that these participants had almost three times the rate of prior neurologic Lyme disease (cranial nerve palsy, neuropathy, meningitis or encephalitis), as abstracted from their medical record, compared to the other two groups. This is consistent with the higher rate of prior intravenous antibiotic treatment in this group as well. We also found that participants in subgroup 3 were significantly more likely to report a co-morbid diagnosis of migraines. In post-hoc analyses, the diagnosis of migraine predated the Lyme disease onset for 80% of those in subgroup 3 with migraine. It is possible that pre-existing neurologic vulnerabilities, such as a history of migraine and/or frank neurologic Lyme disease, are associated with a post-treatment phenotype that encompasses an increase in mood-related symptoms.[39] Although, per the IDSA case definition, we excluded participants with major psychiatric illness, Lyme disease has been associated with a range of neurologic and neuropsychiatric symptoms.[40] Strikingly, although female gender[41,42] and greater exposure to prior stressful life events[43] have both been associated with higher mood symptoms in a number of studies, we did not observe that these participants were any more likely to report heightened mood-related symptoms when faced with similar physical symptom levels.

Our study does have limitations. We ensured greater specificity of our findings to patients whose current illness is more evidently linked to *B. burgdorferi* exposure by operationalizing a narrow research definition of PTLD as eligibility criteria for inclusion into our sample. However, this specificity may also limit generalizability of our findings to a larger population of patients with persistent symptoms following treatment for Lyme disease, especially atypical early

presentations not meeting CDC criteria. It is possible that different eligibility criteria, or different patient samples drawn from other regions of the United States, may have different results. Given the relatively high median household income of our sample, which may have resulted from the geographic location and specialty referral-based nature of our clinic, it will also be important to understand if our findings are generalizable across a broader income range. Furthermore, we relied upon self-report symptom data for these analyses, which is subject to response bias as well as individual variation in perception of symptom severity.[44]

Finally, when applying EFA, Pearson's correlation was used for data from a 4-point Likert scale, which does not satisfy the assumption of a multivariate normal distribution. A nonconvergence issue prevented us from using the more appropriate polychoric correlation. This could lead to spurious multidimensionality and biased factor loadings.[45] However, EFA conceptually met the needs of our research aim, and the results based on Pearson's correlation matrix exhibited meritorious factorability and produced results with satisfactory performance measures. We also followed recommendations to improve our EFA for ordinal data,[46] such as using parallel analysis-based methods for factor retention decision and oblique rotation method. In addition, the main structure of the EFA results is largely consistent with an exploratory symptom clustering analysis we conducted using Kendall's Tau-b, which is nonparametric and is appropriate for ordinal variables.

Reproducibility of the subgroup analysis may be affected by necessary methodological decisions made during the analytic process, including; the scale of the data, the inclusion of a large number of symptoms in the analysis, and the statistical and clinical criteria used during the model selection process. However, the approaches we employed were chosen to achieve as high a degree of theoretical soundness and feasibility as possible. These approaches, in conjunction with the relatively large sample of participants with PTLD that we were able to draw upon for this analysis, allowed for clear and concise interpretability of data.

This analysis represents one of the first to identify and characterize potentially clinically relevant patient subgroups in PTLD. This is important as it may serve as an initial step towards engaging with the heterogeneity in symptom reporting that has long been observed among patients with this condition. Furthermore, in the future it may lead to more targeted interventions or other novel treatment approaches to address the varied and/or multiple factors which contribute to illness perpetuation in PTLD.

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AUTHOR CONTRIBUTIONS

AWR, TY, and JNA all contributed to the conception and design of this study. TY and AWR conducted the data management and statistical analyses. AWR, TY, and JNA drafted, revised, and gave final approval of the manuscript for publication.

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COMPETING INTERESTS

None to declare for any of the authors.

DATA SHARING

De-identified participant data are available upon reasonable request to the corresponding author.

FIGURES

Figure 1. Exploratory factor analysis of 30 common PTLD symptoms suggests a 6-factor model. Three of the symptoms did not load and were dropped in the final model.

Figure 2. Three subgroups of participants identified based on latent profile analysis (panels A and B).

Figure 3. Participant subgroup differences in median standardized symptom factor-based scores, depicted as a heat map. The higher the score, the higher the severity of reported symptoms within each factor.

Figure 4. SF-36 health-related quality of life physical and mental component scores[20] for the three patient subgroups. ns = Not Significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

REFERENCES

1 Steere AC, Strle F, Wormser GP, *et al.* Lyme borreliosis. *Nat Rev Dis Prim* 2016;**2**:16090. doi:10.1038/nrdp.2016.90

2 Stone BL, Tourand Y, Brissette CA. Brave new worlds: the expanding universe of Lyme disease. Vector-Borne Zoonotic Dis. 2017;**17**:619–29. doi:10.1089/vbz.2017.2127

3 Wormser GP, Dattwyler RJ, Shapiro ED, *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**:1089–134. doi:10.1086/508667

4 Wormser GP. Hematogenous dissemination in early Lyme disease. *Wien Klin Wochenschr* 2006;**118**:634–7. doi:10.1007/s00508-006-0688-9

5 Rebman AW, Aucott JN. Post-treatment Lyme disease as a model for persistent symptoms in Lyme disease. *Front Med* 2020;**7**. doi:10.3389/fmed.2020.00057

6 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**:575. doi:10.1136/bmj.38933.585764.AE

7 Nath A. Long-Haul COVID. Neurology. 2020;**95**:559–60. doi:10.1212/WNL.0000000000010640

8 Wormser GP. IDSA Treatment Guidelines for Lyme Disease : Panel Presentation. 2006.

9 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* 2017;**4**:224. doi:10.3389/fmed.2017.00224

10 Aucott JN. Posttreatment Lyme Disease Syndrome. *Infect Dis Clin North Am* 2015;**29**:309–23. doi:10.1016/j.idc.2015.02.012

11 Lobraico J, Butler A, Petrini J, *et al.* New insights into stages of Lyme disease symptoms from a novel hospital-based registry. *J Prim Care Community Heal* 2014;**5**:284–7. doi:10.1177/2150131914540693

12 Zomer TP, Barendregt JNM, van Kooten B, *et al.* Non-specific symptoms in adult patients referred to a Lyme centre. *Clin Microbiol Infect* 2019;**25**:67–70. doi:10.1016/j.cmi.2018.09.016

13 Klempner MS, Hu LT, Evans J, *et al.* Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *N Engl J Med* 2001;**345**:85–92. doi:10.1056/NEJM200107123450202

14 Mukherjee S, Mez J, Trittschuh EH, *et al.* Genetic data and cognitively defined late-onset Alzheimer's disease subgroups. *Mol Psychiatry* Published Online First: 4 December 2018. doi:10.1038/s41380-018-0298-8

15 Curtis C, Shah SP, Chin S-F, *et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;**486**:346–52. doi:10.1038/nature10983

16 Bellato E, Marini E, Castoldi F, *et al.* Fibromyalgia Syndrome: Etiology, Pathogenesis, Diagnosis, and Treatment. *Pain Res Treat* 2012;**2012**. doi:10.1155/2012/426130

17 Centers for Disease Control and Prevention. Lyme disease (Borrelia burgdorferi) 2011 case definition. https://wwwn.cdc.gov/nndss/conditions/lyme-disease/case-definition/2011/

18 Beck AT, Steer RA, Brown GK. *Beck Depression Inventory - Second Edition Manual*. San Antonio: : The Psychological Corporation 1996.

19 Storch EA, Roberti JW, Roth DA. Factor structure, concurrent validity, and internal consistency of the Beck Depression Inventory-Second Edition in a sample of college students. *Depress Anxiety* 2004;**19**:187–9. doi:10.1002/da.20002

20 Ware JE, Kosinski M, Dewey JE. *How to score version 2 of the SF-36 health survey*. Lincoln, RI: : Quality Metric Inc. 2000.

21 Gray MJ, Litz BT, Hsu JL, *et al.* Psychometric properties of the life events checklist. *Assessment* 2004;**11**:330–41. doi:10.1177/1073191104269954

22 Lorig KR, Sobel DS, Ritter PL, *et al.* Effect of a self-management program on patients with chronic disease. *Eff Clin Pr* 2001;**4**:256–62.http://www.ncbi.nlm.nih.gov/pubmed/11769298

23 Ritter PL, Lorig K. The English and Spanish Self-Efficacy to Manage Chronic Disease Scale measures were validated using multiple studies. *J Clin Epidemiol* 2014;**67**:1265–73. doi:10.1016/j.jclinepi.2014.06.009

24 John OP, Naumann LP, Soto CJ. Paradigm shift to the integrative Big Five Trait taxonomy: History, measurement, and conceptual issues. In: John OP, Robins RW, Pervin LA, eds. *Handbook of personality: Theory and research*. New York, NY: : Guilford Press 2008. 114–58. doi:10.1016/S0191-8869(97)81000-8

25 John OP, Donahue EM, L KR. The Big Five Inventory--Versions 4a and 54. Berkeley, CA: 1991. doi:10.1016/S0092-6566(03)00046-1

26 Benet-Martínez V, John OP. Los Cinco Grandes Across Cultures and Ethnic Groups: Multitrait Multimethod Analyses of the Big Five in Spanish and English. *J Pers Soc Psychol* 1998;**75**:729–50. doi:10.1037/0022-3514.75.3.729

27 Martina IS, van Koningsveld R, Schmitz PI, *et al.* Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. *J Neurol Neurosurg Psychiatry* 1998;**65**:743–7.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2170371/pdf/v065p00743.pdf

28 Briggs NE, MacCallum RC. Recovery of weak common factors by maximum likelihood and ordinary least squares estimation. *Multivariate Behav Res* 2003;**38**:25–56. doi:10.1207/S15327906MBR3801_2

29 Storey J, Bass A, Dabney A, *et al.* qvalue: Q-value estimation for false discovery rate control. R package version 2.22.0. 2020.http://github.com/jdstorey/qvalue

30 Centers for Disease Control and Prevention. Lyme Disease Maps: Most Recent Year.

https://www.cdc.gov/lyme/datasurveillance/maps-recent.html (accessed 28 Aug 2019).

31 Fallon BA, Zubcevik N, Bennett C, *et al.* The General Symptom Questionnaire-30 (GSQ-30): a brief measure of multi-system symptom burden in Lyme disease. *Front Med* 2019;**6**:283. doi:10.3389/fmed.2019.00283

32 Safiri S, Kolahi A-A, Hoy D, *et al.* Global, regional, and national burden of neck pain in the general population, 1990-2017: systematic analysis of the Global Burden of Disease Study 2017. *BMJ* 2020;**368**:m791. doi:10.1136/bmj.m791

33 Batheja S, Nields JA, Landa A, *et al.* Post-treatment Lyme syndrome and central sensitization. *J Neuropsychiatry Clin Neurosci* 2013;**25**:176–86. doi:10.1176/appi.neuropsych. 12090223

34 Muris P. Relationships between self-efficacy and symptoms of anxiety disorders and depression in a normal adolescent sample. *Pers Individ Dif* 2002;**32**:337–48. doi:10.1016/S0191-8869(01)00027-7

35 Shnek ZM, Foley FW, LaRocca NG, *et al.* Helplessness, self-efficacy, cognitive distortions, and depression in multiple sclerosis and spinal cord injury. Ann. Behav. Med. 1997;**19**:287–94. doi:10.1007/BF02892293

36 Galvez-Sánchez CM, Montoro CI, Duschek S, *et al.* Depression and trait-anxiety mediate the influence of clinical pain on health-related quality of life in fibromyalgia. *J Affect Disord* 2020;**265**:486–95. doi:10.1016/j.jad.2020.01.129

37 Torres X, Bailles E, Valdes M, *et al.* Personality does not distinguish people with fibromyalgia but identifies subgroups of patients. *Gen Hosp Psychiatry* 2013;**35**:640–8. doi:10.1016/j.genhosppsych.2013.07.014

38 Van Liew C, Leon G, Neese M, *et al.* You get used to it, or do you: symptom length predicts less fibromyalgia physical impairment, but only for those with above-average self-efficacy. *Psychol Heal Med* 2019;**24**:207–20. doi:10.1080/13548506.2018.1524152

39 Affaitati G, Costantini R, Tana C, *et al.* Co-occurrence of pain syndromes. *J Neural Trans* 2020;**127**:625–46. doi:doi: 10.1007/s00702-019-02107-8.

40 Fallon BA, Nields JA. Lyme disease: a neuropsychiatric illness. *Am J Psychiatry* 1994;**151**:1571–83.http://www.ncbi.nlm.nih.gov/pubmed/7943444

41 McLean CP, Asnaani A, Litz BT, *et al.* Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res* 2011;**45**:1027–35. doi:10.1016/j.jpsychires.2011.03.006

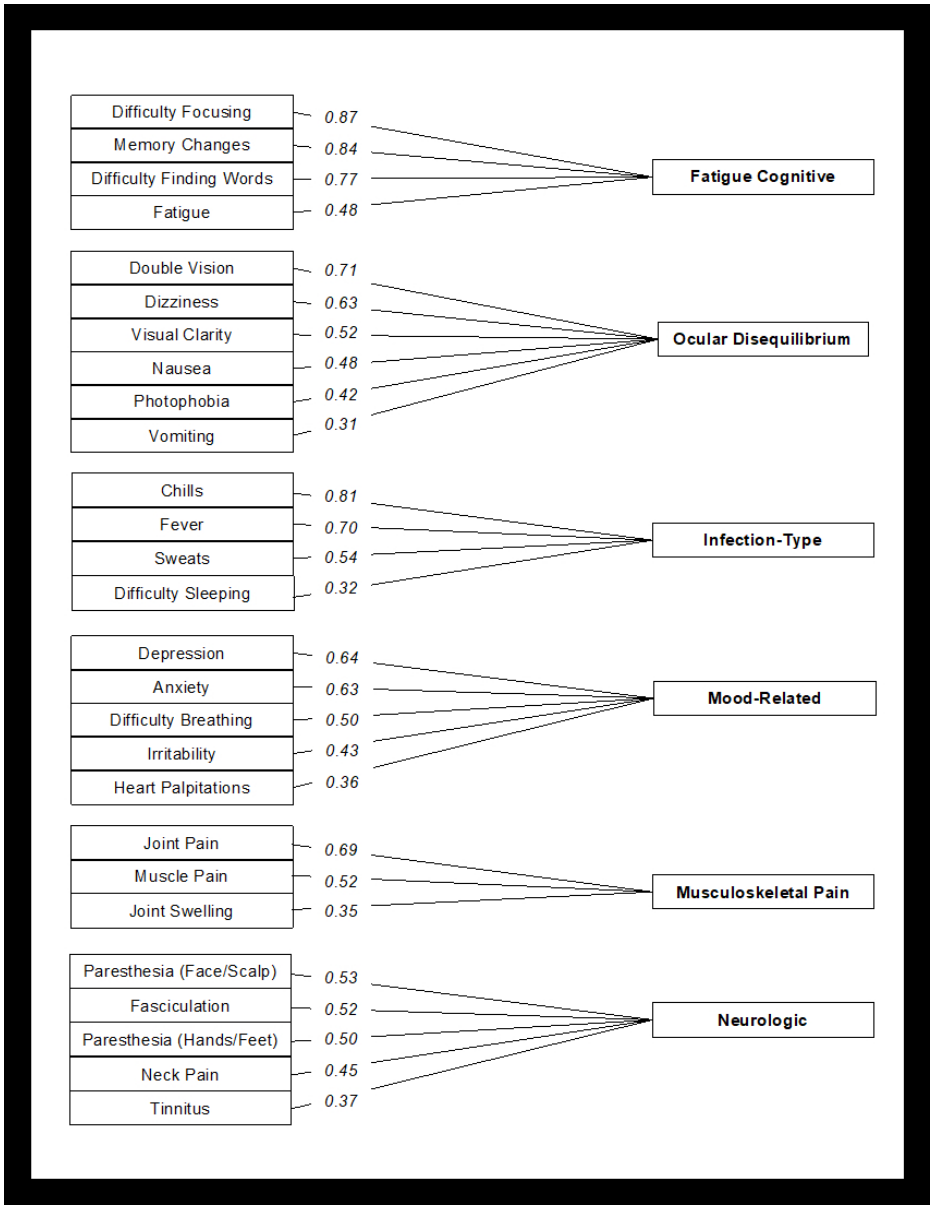
42 Albert PR. Why is depression more prevalent in women? J. Psychiatry Neurosci. 2015;**40**:219–21. doi:10.1503/jpn.150205

43 Tennant C. Life events, stress and depression: a review of recent findings. *Aust N Z J Psychiatry* 2002;**36**:173–82. doi:10.1046/j.1440-1614.2002.01007.x

44 Broadbent E, Petrie KJ. Symptom perception. In: Ayers S, Baum A, McManus C, *et al.*, eds. *Cambridge Handbook of Psychology, Health and Medicine, Second Edition*. Cambridge University Press 2007. 219–23. doi:10.1017/CBO9780511543579.048

45 Bernstein IH, Teng G. Factoring Items and Factoring Scales Are Different: Spurious Evidence for Multidimensionality Due to Item Categorization. *Psychol Bull* 1989;**105**:467–77. doi:10.1037/0033-2909.105.3.467

46 Baglin J. Improving Your Exploratory Factor Analysis for Ordinal Data: A Demonstration Using FACTOR. *Pract Assessment, Res Eval* 2019;**19**. doi:https://doi.org/10.7275/dsep-4220



Exploratory factor analysis of 30 common PTLD symptoms suggests a 6-factor model. Three of the symptoms did not load and were dropped in the final model.

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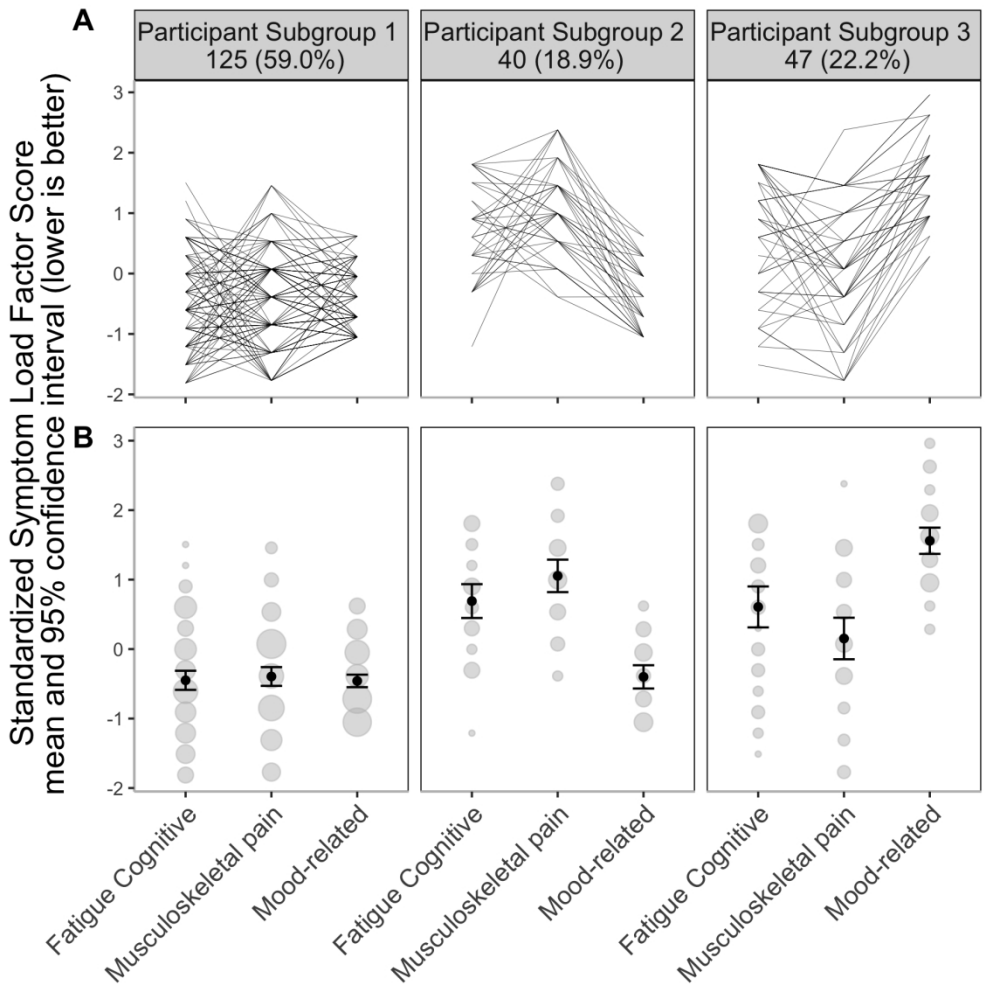

Three subgroups of participants identified based on latent profile analysis (panels A and B).

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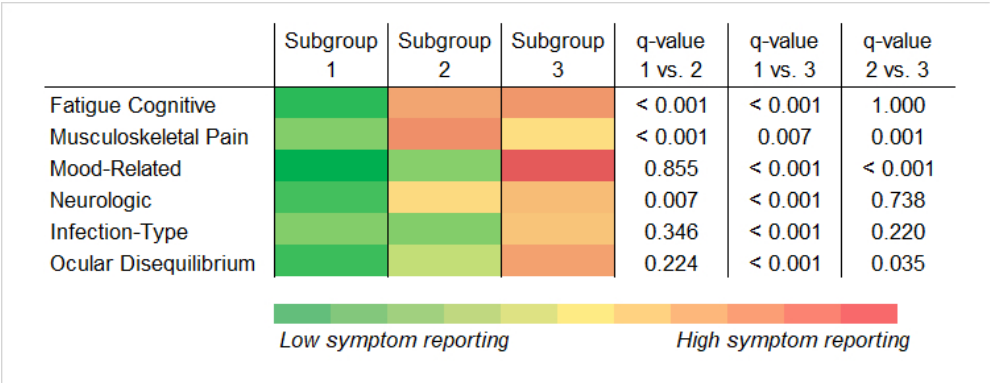

Participant subgroup differences in median standardized symptom factor-based scores, depicted as a heat map. The higher the score, the higher the severity of reported symptoms within each factor.

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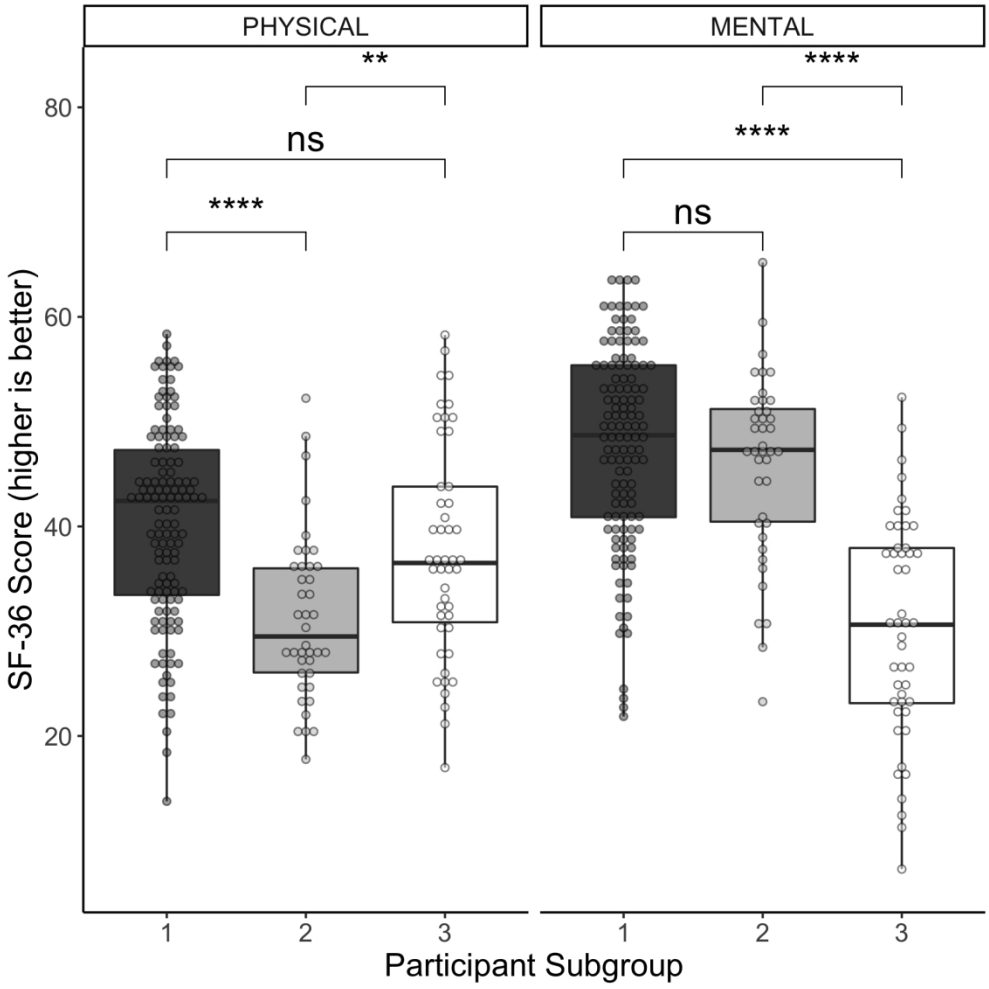

SF-36 health-related quality of life physical and mental component scores[18] for the three patient subgroups. ns = Not Significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

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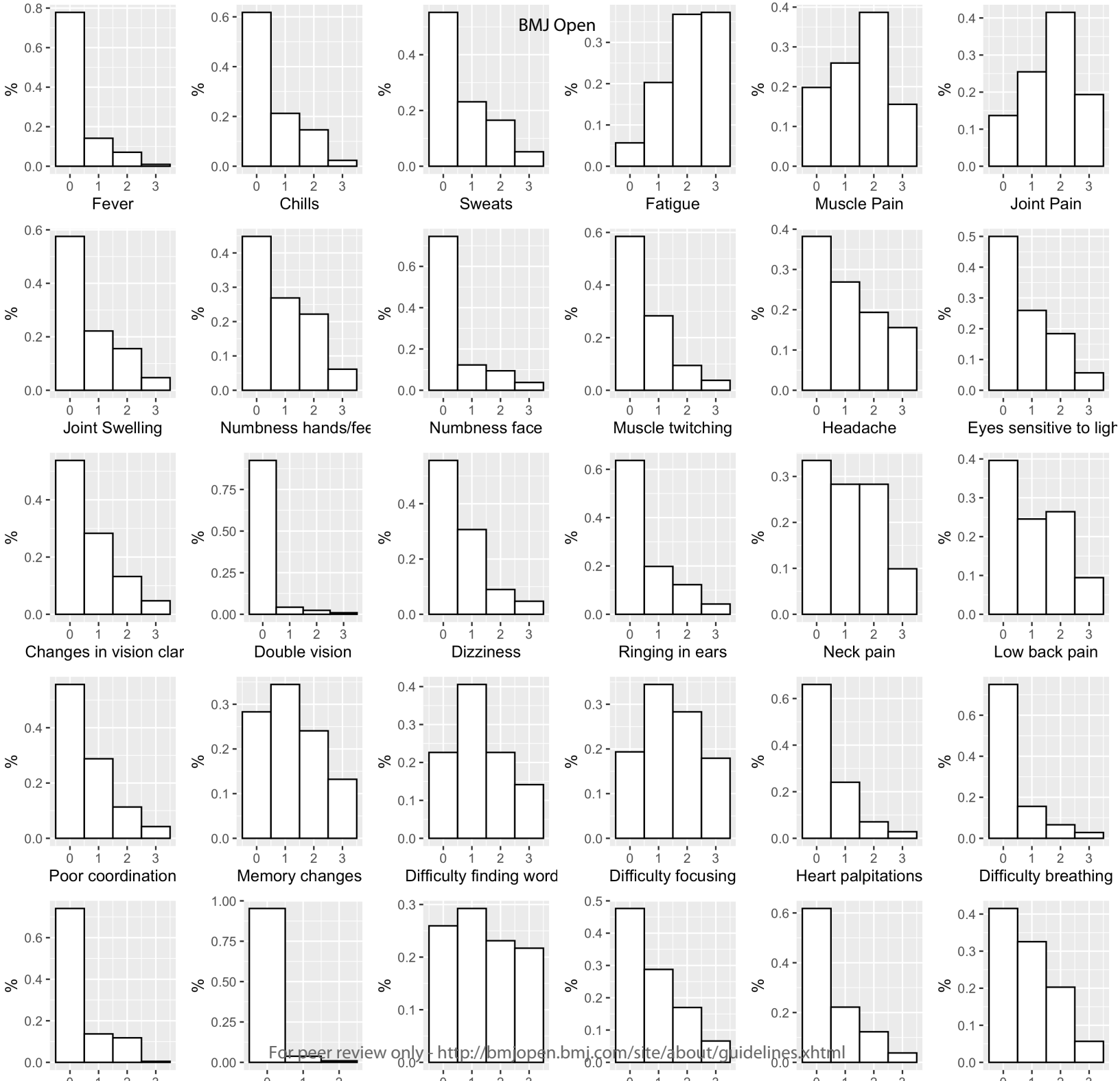

Supplemental Table S1. Exploratory Factor Analysis Loading Matrix

	Fatigue Cognitive	Ocular Disequilibrium	Infection-type	Mood-related	Musculoskeletal pain	Neurologic	*Max loading*	*Second largest loading*	*Difference between max and second largest loading*
Fever	0.05	-0.07	0.70	-0.17	0.02	-0.06	*0.70*	*0.05*	*0.65*
Chills	-0.07	0.06	0.81	0.08	-0.04	0.07	*0.81*	*0.08*	*0.73*
Sweats	0.07	0.06	0.54	0.01	0.15	-0.11	*0.54*	*0.15*	*0.39*
Fatigue	0.48	0.00	0.12	0.15	0.25	-0.04	*0.48*	*0.25*	*0.23*
Muscle Pain	0.13	-0.02	0.09	0.09	0.52	0.20	*0.52*	*0.2*	*0.32*
Joint Pain	0.11	0.00	0.06	-0.09	0.69	0.14	*0.69*	*0.14*	*0.55*
Joint Swelling	-0.07	0.14	0.06	-0.13	0.35	0.05	*0.35*	*0.14*	*0.21*
Numbness hands/feet	-0.02	0.18	0.00	-0.10	0.14	0.50	*0.50*	*0.18*	*0.32*
Numbness face	-0.06	0.21	-0.01	-0.07	0.02	0.53	*0.53*	*0.21*	*0.32*
Muscle twitching	0.01	-0.02	0.05	0.11	0.19	0.52	*0.52*	*0.19*	*0.33*
Headache	0.22	0.21	0.11	0.14	-0.02	-0.01	*0.22*	*0.21*	*0.01*
Eyes sensitive to light	0.05	0.42	0.12	0.15	0.06	0.16	*0.42*	*0.16*	*0.26*
Changes in vision clarity	-0.02	0.52	0.16	0.10	-0.04	0.14	*0.52*	*0.16*	*0.36*
Double vision	0.03	0.71	-0.01	-0.15	-0.06	0.03	*0.71*	*0.03*	*0.68*
Dizziness	0.09	0.63	0.01	0.12	0.05	0.05	*0.63*	*0.12*	*0.51*
Ringing in ears	0.01	0.01	0.15	0.12	-0.06	0.37	*0.37*	*0.15*	*0.22*
Neck pain	0.22	-0.10	-0.01	0.06	0.13	0.45	*0.45*	*0.22*	*0.23*
Low back pain	0.24	-0.01	0.04	0.05	0.33	0.13	*0.33*	*0.24*	*0.09*
Poor coordination	0.32	0.32	-0.03	0.07	0.32	-0.06	*0.32*	*0.32*	*0.00*
Memory changes	0.84	0.05	0.04	-0.04	0.02	-0.06	*0.84*	*0.05*	*0.79*
Difficulty finding words	0.77	0.03	-0.05	0.08	0.05	0.01	*0.77*	*0.08*	*0.69*
Difficulty focusing	0.87	0.00	0.00	0.01	-0.03	0.07	*0.87*	*0.07*	*0.80*
Heart palpitations	-0.03	0.24	-0.03	0.36	0.25	-0.09	*0.36*	*0.25*	*0.11*
Difficulty breathing	-0.17	0.08	0.12	0.50	0.35	0.00	*0.50*	*0.35*	*0.15*

Nausea	0.03	0.48	0.10	0.20	0.04	-0.07	*0.48*	*0.2*	*0.28*
Vomiting	0.15	0.31	0.16	-0.07	-0.07	-0.17	*0.31*	*0.16*	*0.15*
Difficulty sleeping	0.10	0.02	0.32	0.17	0.21	0.06	*0.32*	*0.21*	*0.11*
Anxiety	0.19	0.10	0.04	0.63	-0.10	0.06	*0.63*	*0.19*	*0.44*
Depression	0.10	0.00	-0.01	0.64	-0.02	0.02	*0.64*	*0.1*	*0.54*
Irritability	0.27	-0.01	0.14	0.43	-0.29	0.22	*0.43*	*0.27*	*0.16*

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*
Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	4-5, 7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	N/A

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	7, included in footnotes for Tables 1 and 2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	N/A
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.