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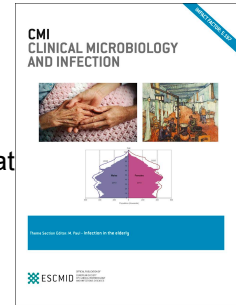
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1   Suspicion of Lyme Borreliosis in Patients Referred to an Infectious Diseases

2   Clinic: what did the patients really have?

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12

13   Running title: Causes of presumed Lyme borreliosis during four-year follow-up in a

14   population-based study

15

16   Category: Original article

17 Abstract

18 Objective: The objective of this study was to evaluate the conditions behind the symptoms

19 in patients with suspected Lyme borreliosis (LB) who were referred to an infectious

20 diseases clinic.

21 Methods: In this retrospective, population-based study, we collected data from the medical

22 records of all patients referred for infectious disease consultations in 2013 because of

23 presumed LB from a population of 1.58 million. The patients were classified according to

24 the certainty of LB based on their symptoms, signs, and laboratory results. Data on the

25 outcomes and subsequent alternative diagnoses during the four-year follow-up period were

26 reviewed from all of the available patient records from public, private, and occupational

27 healthcare providers.

28 Results: A total of 256 patients (16/100 000) were referred due to LB suspicion; 30/256

29 (12%) were classified with definite, 36/256 (14%) with probable, and 65/256 (25%) with

30 possible LB. LB was unlikely in 121/256 (47%) patients. A novel diagnosis was

31 discovered in the background symptoms in 73/256 (29%) of the patients. Previously

32 diagnosed comorbidities caused at least some of the symptoms in 48/256 (19%) of the

33 patients. Other explanations for symptoms were found in 81/121 (67%) of unlikely and

34 22/65 (34%) of possible LB patients. The spectrum of conditions behind the symptoms was

35 very broad and most often musculoskeletal, neurological, psychological, or functional

36 disorders.

37 Conclusions: LB was unlikely in half of the patients with presumed LB. In most cases, the

38 patients had other conditions that explained their symptoms.

39 Keywords: Lyme disease, Lyme borreliosis, *Borrelia burgdorferi*, differential diagnostics,
40 Finland

41   Introduction

42   Diagnosing Lyme borreliosis (LB) can be challenging. The most typical presentation is

43   erythema migrans (EM), which can be confused with erysipelas, cellulitis, tick bite

44   hypersensitivity reaction, or tinea corporis [1-3]. Early dissemination can cause non-

45   specific symptoms such as myalgia, arthralgia, fatigue, fever, and headache, which may

46   appear also in viral infection [4]. Neurological complaints are present in 3-12% of patients

47   with LB [5]. The most common neurological manifestation in Europe is painful

48   lymphocytic meningoradiculitis with or without cranial nerve palsy [6-8]. In late Lyme

49   neuroborreliosis (LNB), defined as an active disease continuing for more than six months,

50   patients may suffer from mononeuropathy, radiculopathy, encephalomyelitis, or cerebral

51   vasculitis [6, 9-14].

52   In addition to typical but sometimes diverse symptoms, diagnosing disseminated LB relies

53   on serological tests and, less frequently, *B. burgdorferi* nucleic acid amplification (NAT)

54   from cerebrospinal fluid (CSF), synovial fluid, or tissue samples [15]. Serological results

55   can be challenging to interpret due to slow antibody response, failure of antibodies to

56   decrease after treatment, and false-positive findings [16]. The diagnosis and treatment of

57   LB have attracted interest among patients and the media. The reliability of recommended

58   two-tier serology tests and physicians' ability to diagnose this disease have been

59   questioned [17].

60   Many patients with medically unexplained symptoms that may indicate LB seek diagnosis

61   and treatment. Overdiagnosis and overtreatment of LB is common, with increased patient

62   morbidity related to unnecessary intravenous and oral antibiotics [18, 19]. The scientific

63   community has recently focused on the accuracy of diagnosing LB and the complexity of

64   irrelevant LB-related antimicrobial treatments [20, 21].

65 The purpose of this study was to evaluate conditions behind the symptoms of presumed

66 disseminated LB among patients with a referral for infectious disease consultation at

67 Helsinki University Hospital in 2013. Hospital District of Helsinki and Uusimaa consists

68 of all hospitals providing specialised health care in southern Finland. All infectious

69 diseases referrals are centralised at Helsinki University Hospital. Local guidelines instruct

70 physicians to refer patients with suspected disseminated LB to our tertiary care hospital.

71 Treatment or diagnostics due to suspected late or disseminated LB are not provided

72 elsewhere in this region. This enabled us to study suspicious disseminated LB in a

73 population-based sample from a catchment area with 1.58 million citizens in an LB-

74 endemic area. [22-24]

75

76 Methods

77 We conducted a retrospective observational population-based study including all adult ($\geq$

78 16 years) patients referred to Helsinki University Hospital due to presumed LB between

79 January 1, 2013, and December 31, 2013. We evaluated patients whose referral led to an

80 appointment at the infectious diseases outpatient clinic or inpatient ward and those whose

81 referral was returned with a written consultation and management suggestions. No

82 exclusion criteria were used. Each patient was identified by their national identity code,

83 which is unique to each resident of Finland. This code was used to identify patients from

84 the various registries. To ensure that all patients with disseminated LB were included in

85 this study, we also searched Helsinki University Hospital's patient database for ICD-10

86 code A69.2 (Lyme borreliosis) to find patients treated at another clinics (for example,

87 rheumatology and neurology).

4

88   All of the patients' medical records were reviewed until the end of 2017 to ascertain their

89   persistent symptoms and subsequent treatment. Medical records were reviewed from

90   Helsinki University Hospital, public and private primary healthcare centres, the patient's

91   occupational health service, and the Finnish Student Health Service in southern Finland.

92   The information collected included gender, age, comorbidities, history of tick bites and

93   erythema migrans, symptoms and signs, symptom duration, laboratory and imaging results,

94   physiological and neurophysiological examinations, number and duration of antibiotic

95   treatments, and novel diagnoses after the referral.

96   After evaluating all of the collected information, the patients were classified into four

97   groups according to the certainty of LB based on the criteria developed for this study

98   (Table 1). All novel diagnoses by the treating physicians were collected from the medical

99   records, including the novel diagnosis for symptoms of presumed LB and other symptoms

100   separately. A previous condition or a novel diagnosis might have explained some

101   symptoms among the patients with definite, probable, or possible LB. Previous conditions

102   were comorbidities that were diagnosed before the referral, but a patient or a treating

103   physician might have sought an additional explanation for their symptoms. Data collection

104   and patient classification was conducted retrospectively by one researcher (EK), a

105   physician who specialised in internal medicine and in difficult classifications it was

106   checked by another physician.

107   The method for defining the patients' *B. burgdorferi*-specific antibody levels was

108   previously described [25] . The first-tier antibody serum tests were *Borrelia afzelii* + VlsE

109   IgG ELISA and *Borrelia afzelii* IgM ELISA (Sekisui Virotech, Rüsselsheim, Germany).

110   These tests were also used for CSF antibody testing, followed by detecting intrathecal

111   antibody production using relative antibody measurement in the serum and CSF. Second-

112   tier serum tests were Liaison *Borrelia* IgG and Liaison *Borrelia* IgM (Liaison *Borrelia* IgG

113   and IgM DiaSorin, Saluggia, Italy). The sum of numeric values from the two EIA tests for

114   IgG (Sekisui Virotech and DiaSorin) was used for categorisation (negative < 25, low

115   concentration 25-59, intermediate 60-179, and high ≥ 180). Antibody level categorisation

116   for high concentrations required a positive immunoblot for IgG. The sum of the IgM

117   concentration ≥ 25 without IgG antibodies was categorised as low total antibody

118   concentration. Intermediate and high antibody levels were regarded as markedly positive in

119   the patient classification according to the certainty of LB. This diagnostic algorithm and

120   interpretation cut-offs have been analytically and clinically validated by the Helsinki

121   University Hospital Laboratory, and further verified by using clinical data. The algorithm,

122   being purely arbitrary, is based on extensive previous experience on these tests.

123   The statistical methods are presented in the supplementary material.

124   This study was approved by the research board of the Inflammation Centre at the Helsinki

125   University Hospital. The usage of the patient records of the municipal health centres,

126   occupational healthcare centres, The Finnish Student Health Service and private healthcare

127   clinics were approved by the Finnish Institute for Health and Welfare. The Social

128   Insurance Institution provided information on all antimicrobial purchases from pharmacies

129   of all of the patients included in this study. Because of the retrospective nature of this

130   study, no ethical approval was necessary.

131

132   Results

133   The total number of patients with a referral due to LB suspicion to Helsinki University

134   Hospital's Infectious Diseases Clinic in 2013 was 256 (16/100 000 population). The search

135   of Helsinki University Hospital's database for ICD-10 code A69.2 did not reveal any LB

136   patients who did not consult an infectious diseases specialist. Among all of the patients

137 with a referral, 167 (65%) were called for a visit at the Infectious Diseases Clinic and 89

138 (35%) referrals were returned with a written consultation reply by an infectious disease

139 specialist to the remitting physician mostly in general practise but also in neurology or

140 rheumatology (Supplementary Table 2). Most (59%) of the referrals were sent between

141 July and November (Supplementary Figure 1).

142 According to the referral and review of the patients charts until the end of 2017, 30

143 (11.7%) and 36 (14.1%) were categorised having definite and probable LB, respectively

144 (Figure 1). The numbers of patients with possible or unlikely LB were 65 (25.4%) and 121

145 (47.3%), respectively. In four (1.6%) patients, the certainty of LB could not be determined

146 due to a lack of sufficient information on the clinical picture and serological tests. These

147 four patients were excluded from the comparison between the groups.

148 The patients' mean age was 53.2 (SD 15.0, range 16-85), and 164 (64%) were female

149 (Table 2). There were more females in the unlikely LB group than definite LB (p = 0.009).

150 The median duration of the symptoms before referral was three months (range 0-520). The

151 duration of the symptoms was longer in the unlikely LB group than the patients with

152 definite or probable LB (p < 0.001 and p = 0.012, respectively). In addition, the patients

153 with definite LB had statistically significantly shorter duration of symptoms than the

154 possible LB (p = 0.002) and probable LB groups (p = 0.006). The comparisons between

155 the groups' baseline characteristics are shown in the supplementary material

156 (Supplementary Table 3).

157 Among the 30 patients with definite LB, 93% had symptoms that could be classified as

158 suggestive of LB based on the literature whereas the proportion of such symptoms were

159 found in 50-52% of the patients in the other groups (Supplementary Tables 1 and 4). The

160 most typical signs and symptoms among the patients with definite LB included facial

161 palsy, radiculitis, and paraesthesia (Table 3). Arthralgia was reported by approximately

162 half of the patients in the other LB certainty groups and was more common than in definite

163 LB. Otherwise, the symptoms were variable with no common denominator (Table 3).

164 A cerebrospinal fluid (CSF) specimen was taken from 115 (45%) patients with normal

165 findings in 77 (67%) of them (Table 4). In the unlikely LB group, 76% of the patients had

166 negative or low antibodies and only 13% of the patients had intermediate or high serum *B.*

167 *burgdorferi* antibody levels. However, low antibody levels were also common in the other

168 groups. The second-tier test was conducted in 188 patients. *B. burgdorferi* NAT was

169 positive in 1/77, 0/3, and 2/4 of the analysed CSF, synovial fluid and skin lesion

170 specimens, respectively.

171 The presumed LB symptoms caused at least one follow-up contact with healthcare after the

172 consultation reply or the initial visit at the Infectious Diseases Clinic in 108 (89%) patients

173 with unlikely LB, 57 (88%) patients with possible LB, 35 (97%) patients with probable

174 LB, 27 (90%) patients with definite LB, and 2 (50%) patients with unknown certainty of

175 LB. Diagnostic conclusions based on the follow-up of 256 patients are presented in Figure

176 1. Alternative novel conditions or diagnoses that partially explained some or majority of

177 the symptoms mentioned in the referral were revealed in 73 patients (28%). In 107 (42%)

178 patients, symptoms were at least partly explained by previous or novel diagnoses, and only

179 31 (12%) patients did not have an obvious reason that could explain their symptoms.

180 Among the patients with unlikely LB, 67% had either a previous condition or a novel

181 diagnosis explaining their symptoms. Previous and novel diagnoses behind the symptoms

182 are presented in Table 5 and separately in the supplementary material (Supplementary

183 Tables 7 and 8).

184

185 Discussion

186 We evaluated the probability of LB and other reasons during a four-year follow-up for the

187 symptoms in 256 patients who were referred to infectious disease specialist consultations

188 due to suspected LB in 2013. Definite or probable LB was diagnosed among 26% of the

189 patients and possible LB in 25% of the patients. LB was unlikely in 47% of the patients.

190 The symptoms varied widely, but the patients classified with unlikely LB had significantly

191 longer symptom duration than those who were classified with definite or probable LB. In

192 42% of the patients, either a previous or novel condition upon 4-year follow-up explained

193 some or all of their original referral symptoms.

194 In 2013, laboratories in the area near Helsinki University Hospital reported 556 serological

195 or NAT findings of *B. burgdorferi* to the National Infectious Disease Registry (NIDR),

196 more than double the number of cases referred to our centre [26]. However, all of the

197 serological findings in the NIDR do not represent disseminated LB, and despite

198 instructions, many specimens were obtained from patients with EM only or from patients

199 without symptoms of LB. Some of the disseminated cases may also have been treated

200 elsewhere.

201 In a previous epidemiological study in Finland, the incidence of LNB was 2.4/100,000 in

202 2011 and the incidence of LA did not exceed 1.0/100,000 in 1996-2014 [23]. We assume

203 that most LNB cases were treated at our hospital, so this led to an LNB (definite and

204 probable) incidence of 2.2/100,000 in 2013. The portion of LNB from laboratory

205 confirmed cases was 6%. LNB was seven times more frequent than Lyme arthritis in our

206 patients even though patients treated in rheumatology clinic were included.

207 The strengths of our study are its population-based approach of disseminated LB, four-year

208 follow-up, and comprehensive access to patient records from several different caregivers.

9

209 Our patient cohort differed from other recent studies concerning confirmation of LB

210 diagnosis [18, 20, 27, 28]. The proportion of definite, probable, and possible LB patients

211 was higher (51%) in our patient cohort than 13-23% in those studies. Furthermore, only

212 20% of our patients had been prescribed previous antimicrobial treatment effective for LB

213 before referral compared to 50-85% in previous studies [18, 20, 27, 28]. This might reflect

214 our local guidelines to refer suspected disseminated LB for an infectious disease specialist

215 consultation.

216 As expected, among the patients with definite or probable LB, only 4/66 (6%) had other

217 conditions that explained their symptoms partly. In patients with possible LB, 34% had

218 another underlying condition causing some of their symptoms. In 67% of patients

219 classified as unlikely LB, other conditions were most likely behind their symptoms. Our

220 data support the notion that when clinical and laboratory judgement demonstrates unlikely

221 LB, other causes of the patient's symptoms should be actively assessed.

222 Causes of symptoms in the patients with unlikely LB were variable but similar to

223 previously reported [18, 27]. Musculoskeletal problems, neurological pathologies, and

224 psychiatric disorders were the most common reasons. In addition, three cases with

225 malignancy were found, which was previously demonstrated as one caveat [29]. Some of

226 our patients needed rapid therapy for their underlying disease, such as coronary artery

227 disease, pneumonia, or cellulitis. Our results amplify the importance of appropriate

228 differential diagnostics among these patients. Frequent and prolonged antimicrobial

229 treatments for suspicion of LB has been associated with adverse events and might delay

230 necessary diagnostic procedures and treatment of underlying causes [19, 30, 31].

231 Our study has some limitations. As in all retrospective studies, the information collected

232 might be incomplete. In our study, this came into question, especially in the documentation

233   of the patients' symptoms and signs. Some of the symptoms might have been dismissed

234   despite thorough patient assessment and review of the patient records from all caregivers

235   that could be contacted. It is also possible that all of the novel diagnoses were not

236   registered in the patient records or were not assessed using the proper criteria. In addition,

237   our population-based setup is based on assumption, that all disseminated forms of LB were

238   treated in our hospital. We did not search data from entire population, leading to the

239   possibility of missing some cases. Also, we did not have the opportunity to follow patients

240   who moved to another region during the follow-up period. The exact number of patients

241   lost to follow-up is unknown, but 89% of the patients had at least one follow-up healthcare

242   contact.

243   In conclusion, only half of the patients with a referral because of suspicion of LB had

244   definite, probable, or possible LB. The patients with unlikely LB had other conditions in

245   67% of cases that explained their symptoms but upon the four-year follow-up, 12% of all

246   referred patients with LB suspicion were not given an explanation for their symptoms.

247

248   Transparency declaration

258 Author contributions

259 A. Järvinen conceived and supervised the study. E. Kortela conducted the data collection

260 and data analyses and wrote the draft manuscript. All of the authors participated in the

261 study design, revised the draft manuscript, and approved the final manuscript.

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263

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343

344

14

345   Table 1. Classification of the patients into groups according to certainty of LB in this study

Criteria for definite LB (criteria 1, 2, or 3 fulfilled)
1. Positive *B. burgdorferi* NAT from CSF, synovial fluid, or skin biopsy together with symptoms suggestive of LB[a]
2. Intrathecal production of *B. burgdorferi*-specific antibodies and CSF pleocytosis (≥ 5 leukocytes/µl) together with suggestive symptoms of LNB[a] without other obvious reasons
3. Seroconversion[b] of *B. burgdorferi* and suggestive symptoms of LB[a] without other obvious reasons

Criteria for probable LB (criteria 1 or 2 and 3 + 4 fulfilled)
1. Markedly positive[c] *B. burgdorferi* antibody levels in serum
2. Typical EM during the previous three months
3. Symptoms suggestive of LB[a] without other obvious reasons
4. Improvement after antimicrobial treatment[d]

Criteria for possible LB (criteria 1 or 2 fulfilled)
1. Symptoms suggestive of LB[a] without other obvious reasons and *B. burgdorferi* specific IgG antibodies in serum[e]
2. In the absence of *B. burgdorferi*-specific antibodies, the duration of symptoms less than two months, specificity of symptoms of LB, and response to antimicrobial treatment[d]

Criteria for unlikely LB (criteria 1, 2, or 3 fulfilled)
1. Absence of *B. burgdorferi* IgG antibodies in the serum or CSF with symptom duration for more than two months
2. Atypical symptoms and failure to respond to antimicrobial treatment[d]
3. Other obvious reasons for symptoms

346

347   LB, Lyme borreliosis; NAT, nucleic acid amplification; CSF, cerebrospinal fluid; LNB, Lyme
348   neuroborreliosis; EM, erythema migrans.

349   [a]Symptoms suggestive of LNB or LB are listed in Supplementary Table 1.

350   [b]Increase in IgG antibodies between concurrently analysed paired serum samples: S-VlsEAbG ≥ 30 units and
351   S-VlsEAbG $\geq 50\%$ units (DiaSorin) together with an increase in S-BorrAbG (Sekisui Virotech).

352   [c]Presented in methods. Intermediate and high antibody levels were regarded as markedly positive.

353   [d]Reported by the patient.

354   [e]Sum of numeric values from two EIA tests (Sekisui Virotech and DiaSorin) was ≥ 25.

355

356

357 Table 2. Baseline characteristics of the patients referred for consultation due to suspicion

358 of LB categorised according to the probability of LB in final analysis after 4-year follow-

359 up of their patient records (n = 256)

	Definite LB n = 30	Probable LB n = 36	Possible LB n = 65	Unlikely LB n = 121	Certainty cannot be determined n = 4
Female gender	13 (36.1%)	21 (58.3%)	39 (56.5%)	89 (73.6%)	2 (50%)
Age in years, mean (SD)	51.8 (16.4)	56.7 (14.2)	55.6 (14.7)	51.1 (15.0)	53.8 (15.2)
Number of coexisting diseases, median (IQR)	1 (0.75-3)	1 (0-3)	1 (1-2.5)	2 (1-3)	1.5 (0.3-2.8)
Tick bite during the past year	9 (30%)	15 (41.7%)	29 (44.6%)	35 (28.9%)	2 (50%)
EM during the past year					
Singular	6 (20%)	18 (50.0%)	13 (20.0%)	15 (12.4%)	1 (25.0%)
Multiple	0	3 (8.3%)	3 (4.6%)	1 (0.8%)	0
Rash atypical for EM	2 (6.7%)	0	7 (10.8%)	15 (12.4%)	1 (25.0%)
Duration of symptoms in months, median (IQR)	1 (0.5-2.1)	3 (1-4.8)	3 (1-10)	6 (2-24)	0 (0-7.5)
Number of symptoms, median (IQR)	3 (2-6)	3 (2-4)	3 (2-4)	3 (2-5)	0 (0)
Principal manifestations according to referral					
No information	0	0	0	1 (0.8%)	1 (25.0%)
Cutaneous	1 (3.3%)	3 (8.3%)	2 (3.1%)	6 (5.0%)	2 (50.0%)
Joint symptoms	0	6 (16.7%)	16 (24.6%)	22 (18.2%)	0
Acute dissemination	3 (10.0%)	15 (41.7%)	22 (33.8%)	25 (20.7%)	0
Neurological symptoms	26 (86.7%)	10 (27.8%)	22 (33.8%)	36 (29.8%)	0
Non-specific symptoms	0	2 (5.6%)	2 (3.1%)	23 (19.0%)	1 (25.0%)
Asymptomatic seropositive	0	0	0	8 (6.6%)	0
Reason for referral was something else	0	0	1 (1.5%)	0	0
Antimicrobial treatment effective for LB before referral[a]	3 (10.0%)	6 (16.7%)	10 (15.4%)	31 (25.6%)	2 (50.0%)
Patient was evaluated at infectious diseases clinic	24 (80.0%)	30 (83.3%)	51 (78.5%)	62 (51.2%)	0

360

361 Data represents number of patients (%) unless otherwise stated. LB, Lyme borreliosis; EM, erythema
362 migrans.

363 [a]Described in the supplementary material.

364

365   Table 3. Symptoms of the patients referred for consultation due to suspicion of LB (n =

366   252)

	Definite LB n = 30	Probable LB n = 36	Possible LB n = 65	Unlikely LB n = 121	P value
Facial nerve palsy	18 (60%)	1 (2.8%)	3 (4.6%)	3 (2.5%)	< 0.001[a]
Diplopia	6 (20%)	0	0	4 (3.3%)	< 0.001[a]
Other peripheral nerve palsy	2 (6.7%)	1 (2.8%)	2 (3.1%)	5 (4.1%)	0.862
Radiculitis	11 (36.7%)	2 (5.6%)	5 (7.7%)	1 (0.8%)	< 0.001[a]
Peripheral neuropathy	2 (6.7%)	0	1 (1.5%)	1 (0.8%)	0.145
Paraesthesia	10 (33.3%)	5 (13.9%)	20 (30.8%)	38 (31.4%)	0.174
Monoarthritis	0	3 (8.3%)	4 (6.2%)	6 (5.0%)	0.487
Oligoarthritis	0	1 (2.8%)	1 (1.5%)	4 (3.3%)	0.875
Headache	9 (30.0%)	10 (27.8%)	17 (26.2%)	43 (35.5%)	0.575
Neck and shoulder pain	5 (16.7%)	5 (13.9%)	5 (7.7%)	11 (9.1%)	0.441
Myalgia	7 (23.3%)	17 (47.2%)	22 (33.8%)	48 (39.7%)	0.202
Arthralgia	3 (10.0%)	20 (55.6%)	36 (55.4%)	52 (43.0%)	< 0.001[a]
Fatigue	9 (30.0%)	18 (50.0%)	26 (40.0%)	47 (38.8%)	0.427
Vertigo	6 (20.0%)	4 (11.1%)	12 (18.5%)	24 (19.8%)	0.721
Hypoacusis	1 (3.3%)	1 (2.8%)	0	3 (2.5%)	0.483
Tinnitus	0	1 (2.8%)	0	2 (1.7%)	0.604
Muscle weakness	3 (10.0%)	5 (13.9%)	3 (4.6%)	22 (18.2%)	0.056
Nausea	4 (13.3%)	1 (2.8%)	4 (6.2%)	11 (9.1%)	0.400
Weight loss	2 (6.7%)	0	2 (3.1%)	10 (8.3%)	0.202
Flu-like symptoms	4 (13.3%)	1 (2.8%)	5 (7.4%)	5 (4.2%)	0.224
Fever more than 38 degrees	6 (20.0%)	6 (16.7%)	4 (6.2%)	10 (8.3%)	0.092
Subjective memory difficulties	4 (13.3%)	1 (2.8%)	3 (4.6%)	10 (8.3%)	0.339
Objective memory difficulty	0	1 (2.8%)	2 (3.1%)	2 (1.7%)	0.707
Confusion	1 (3.3%)	0	0	1 (0.8%)	0.384
Heart conduction system disturbances	0	2 (5.6%)	0	0	0.034[a]
Arrhythmia	0	0	2 (3.1%)	4 (3.3%)	0.762
Iritis or conjunctivitis	0	2 (5.6%)	4 (6.2%)	2 (1.7%)	0.190
Disturbances in vision	4 (13.3%)	1 (2.8%)	5 (7.7%)	6 (5.0%)	0.297
Symptoms appear as episodes	2 (6.7%)	0	3 (4.6%)	12 (9.9%)	0.088

367   Patients with unknown certainty are not reported here because of the lack of information about symptoms and

368   the small number in the group. LB, Lyme borreliosis.

369   [a]Further comparison between the groups is presented in Supplementary Table 5.

370

371 Table 4. Diagnostic procedures of the patients consulted for suspicion of LB (n = 252)

	Definite LB n = 30	Probable LB n = 36	Possible LB n = 65	Unlikely LB n = 121
B.b antibody levels in serum[a]				
Not enough information	1 (3.3%)	1 (2.8%)	6 (9.2%)	12 (9.9%)
Negative	0	0	3 (4.6%)	36 (29.8%)
Low positive	1 (3.3%)	6 (16.7%)	22 (33.8%)	56 (46.3%)
Intermediate	19 (63.3%)	14 (38.9%)	26 (40.0%)	12 (9.9%)
High positive	5 (16.7%)	15 (41.7%)	8 (12.3%)	4 (3.3%)
Seroconversion	4 (13.3%)	0	0	1 (0.8%)
CSF sampling	n = 29 (96.7%)	n = 9 (25.0%)	n = 33 (50.8%)	n = 44 (36.4%)
Normal	2 (6.9%)	7 (77.8%)	27 (81.8%)	41 (93.2%)
Pleocytosis, normal AI	0	1 (11.1%)	4 (12.1%)	3 (6.8%)
No pleocytosis, positive AI	0	1 (11.1%)	2 (6.1%)	0
Pleocytosis and positive AI	27 (93.1%)	0	0	0
ENMG	n = 5 (16.7%)	n = 2 (5.6%)	n = 8 (12.3%)	n = 18 (14.9%)
No information				1 (5.6%)
Normal	1 (20.0%)	1 (50.0%)	2 (25.0%)	10 (55.6%)
Abnormal[b]	4 (80.0%)	1 (50.0%)	6 (75.0%)	7 (38.9%)
EEG	n = 1 (3.3%)		n = 1 (1.5%)	n = 5 (4.1%)
Normal	0	0	0	3 (60.0%)
Abnormal[b]	1 (100%)	0	1 (100%)	2 (40.0%)
Brain MRI	n = 11 (36.7%)	n = 2 (5.6%)	n = 7 (10.8%)	n = 22 (18.2%)
Unknown result	1 (9.1%)	0	0	1 (4.5%)
Normal	3 (27.3%)	2 (100%)	6 (85.7%)	19 (86.4%)
Abnormal[b]	7 (63.6%)	0	1 (14.3%)	2 (9.1%)
Brain MRI + MRA (with normal findings)	0	0	1 (1.5%)	2 (1.7%)
Cervical spine MRI	n = 1 (3.3%)			n = 4 (3.3%)
Normal	0	0	0	0
Abnormal[b]	1 (100%)	0	0	4 (100%)
Lumbar spine MRI	n = 1 (3.3%)		n = 1 (1.5%)	n = 3 (2.5%)
Normal	1 (100%)	0	1 (100%)	2 (66.7%)
Abnormal[b]	0	0	0	1 (33.3%)
Whole spinal column MRI	n = 2 (6.7%)			n = 2 (1.7%)
Normal	0	0	0	2 (100%)
Abnormal[b]	2 (100%)	0	0	0
Foot MRI with abnormal result	0	0	0	1 (0.8%)
Neuropsychological tests	n = 1 (3.3%)	n = 1 (2.8%)	n = 2 (3.1%)	n = 11 (9.1%)
Normal	0	0	0	3 (27.3%)
Slightly abnormal[b]	1 (100%)	0	1 (50.0%)	6 (54.5%)
Abnormal[b]	0	1 (100%)	1 (50.0%)	2 (18.2%)

372 Abbreviations: LB, Lyme borreliosis; *B.b, Borrelia burgdorferi*; CSF, cerebrospinal fluid; AI, (anti-*Borrelia*)

373 antibody index; ENMG, electroneuromyography; EEG, electroencephalogram; MRI, magnetic resonance

374 imaging; MRA, magnetic resonance angiography.

375 Data are number of patients (%). These diagnostic procedures were performed mainly between autumn 2012

376 and spring 2014.

18

377   ᵃSum of numeric values from the two EIA tests for IgG (Sekisui Virotech and DiaSorin) was used for

378   categorisation (negative < 25, low positive 25-59, intermediate 60-179, high ≥ 180, and additionally positive

379   immunoblot for IgG). The sum of numeric IgM concentration ≥ 25 without IgG antibodies was categorised as

380   a low total antibody concentration. The data from *Borrelia* IgG and IgM immunoblots are presented in

381   Supplementary Table 6.

382   ᵇIncluding all kinds of abnormal changes not specific to LB.

383   Table 5. Reasons for symptoms (novel diagnoses and previous conditions)

	Diagnosis (n = 177)
Musculoskeletal problems	**60 (33.9%)**
Degenerative spinal disease	19
Osteoarthritis	18
Degenerative tendinopathy (like rotator cuff injury)	8
Non-specific musculoskeletal pain	8
Overuse injuries (for example, tennis elbow)	5
Ulnar nerve compression	1
Hypermobility syndrome	1
Neurological pathologies	**22 (12.4%)**
Tension-type headache	5
Migraine	5
Parkinson`s disease	2
Normal pressure hydrocephalus	2
Demyelination in the central nervous system	1
Alzheimer's disease	1
Morton neuroma	1
Small fibre neuropathy	1
Axial myopathy	1
Amyotrophic lateral sclerosis	1
Non-specific cervical syringomyelia	1
Multiple sclerosis	1
Psychiatric disorders	**18 (10.2%)**
Depression	7
Somatic symptom disorder	3
Alcohol use disorder	2
Post-traumatic stress disorder	1
Attention deficit hyperactivity disorder	1
Insomnia non-organic	1
Panic disorder	1
Personality disorder	1
Non-specific dissociative disorder	1
Functional disorders	**15 (8.5%)**
Fibromyalgia	11
Chronic fatigue syndrome	3
Hypersomnia	1
Rheumatological diseases	**14 (7.9%)**
Reactive arthritis	4
Rheumatoid arthritis	3
Oligoarthritis seronegative	2
Ankylosing spondylitis	1
CREST syndrome	1
Sjögren's syndrome	1
Psoriatic arthritis	1
Polymyalgia rheumatica	1
Other infectious diseases	**9 (5.1%)**
Meningoencephalitis of unknown origin	2
Tick-borne encephalitis	1
Syphilis	1
Cellulitis	1
Pneumonia	1
Sinusitis	1
Cytomegalovirus infection	1
Chronic hepatitis C virus infection	1
Dermatological diseases	**9 (5.1%)**
Lymphocytoma cutis	2
Psoriasis	1

Erythema annulare	1
Eczema nummular	1
Erythema chronicum migrans st post	1
Atopic dermatitis	1
Granuloma annulare	1
Capillary malformations (port wine stains)	1
Otorhinolaryngological diseases	**8 (4.5%)**
Benign positional vertigo	3
Bell's palsy	3
Sensorineural hearing loss	1
Sialadenitis	1
Gastroenterological diseases	**5 (2.8%)**
Chronic *H. pylori* gastritis	2
Celiac disease	1
Alcoholic liver cirrhosis	1
Gastroesophageal reflux disease	1
Ophthalmological diseases	**4 (2.3%)**
Retinal detachment	2
Dry eyes	1
Iritis	1
Cardiovascular diseases	**4 (2.3%)**
Coronary artery disease	1
Atrial fibrillation	2
Chronic peripheral venous insufficiency	1
Cancer	**3 (1.7%)**
Tonsillar cancer	1
Ovarian cancer and peritoneal carcinomatosis	1
Basal cell carcinoma	1
Others	**6 (3.4%)**
Sleep apnoea	3
Idiopathic angio-oedema	1
Hypophysitis with panhypopituitarism	1
Previous brain injury	1

384 Data are number of new diagnoses (%).

385

386   Figure 1. Classification of patients referred due to suspicion of Lyme borreliosis (LB)

387   according to the certainty of LB and number of patients with previous and novel diagnoses

388   or conditions revealed in follow-up from the patient records that could explain their

389   symptoms behind referral.

390

391   [*]Explains part of the symptoms. LB, Lyme borreliosis; EM, erythema migrans; LNB,

392   Lyme neuroborreliosis.

256 patients with a referral because of LB suspicion

4 patients with unknown certainty of LB

30 definite LB:

1 EM
2 disseminated LB
27 LNB

36 probable LB:

3 EM
21 disseminated LB
7 LNB
5 Lyme arthritis

65 possible LB:

2 skin manifestations
12 neurologic symptoms
5 arthritis

121 unlikely LB

31 without diagnosis

9 asymptomatic

1 previous diagnosis*

1 novel diagnosis*

2 novel diagnosis*

9 previous diagnosis*

13 novel diagnosis*

24 previous conditions

43 novel diagnosis

14 previous conditions + novel diagnosis