ORIGINAL ARTICLE INFECTIOUS DISEASES

Ticking the right boxes: classification of patients suspected of Lyme borreliosis at an academic referral center in the Netherlands

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Abstract

To provide better care for patients suspected of having Lyme borreliosis (LB) we founded the Amsterdam Multidisciplinary Lyme borreliosis Center (AMLC). The AMLC reflects a collaborative effort of the departments of internal medicine/infectious diseases, rheumatology, neurology, dermatology, medical microbiology and psychiatry. In a retrospective case series, characteristics of 200 adult patients referred to the AMLC were recorded, and patients were classified as having LB, post-treatment LB syndrome (PTLBS), persistent *Borrelia burgdorferi sensu lato* (*s.l.*) infection despite antibiotic treatment or no LB. In addition, LB, PTLBS and persistent *B. burgdorferi s.l.* infection cases were classified as 'definite,' 'probable' or 'questionable.' Of the 200 patients, 120 (60%) did not have LB and 31 (16%) had a form of localized or disseminated LB, of which 12 were classified as definite, six as probable and 13 as questionable. In addition, 34 patients (17%) were diagnosed with PTLBS, of which 22 (11%) were probable and 12 (6%) questionable. A total of 15 patients (8%) were diagnosed with persistent *B. burgdorferi s.l.* infection, of which none was classified as definite, three as probable and 12 as questionable. In conclusion, in line with previous studies, the number of definite and probable (persisting) LB cases was low. The overall high number of questionable cases illustrates the fact that it can sometimes be challenging to either rule out or demonstrate an association with a *B. burgdorferi s.l.* infection, even in an academic setting. Finally, we were able to establish alternative diagnoses in a large proportion of patients.

Keywords: Chronic disease, Lyme disease, multidisciplinary communications, retrospective studies, tertiary care centers
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Introduction

Lyme borreliosis (LB) is the most common tick-borne disease in the northeastern part of the United States and in Europe in temperate climate zones [1]. LB is caused by spirochetes of the *Borrelia burgdorferi sensu lato* (*s.l.*) group [2]. In the Netherlands, the number of LB cases appears to be on the rise, from 100 per 100 000 inhabitants in 2005 to 134 per 100 000 inhabitants in 2009 [3]. Similarly, the number of visits to Dutch general practitioners (GPs) for tick bites rose from 371 per 100 000 in 2001 to 446 and 564 in 2005 and 2009, respectively. Recently, the Dutch ministry of health has asked for concerted action on ticks and LB and has asked for the development of a nationwide collaborative effort between medical and scientific institutes focusing on LB to improve LB care and research in the Netherlands.

Diagnosis and treatment of early localized LB in the Netherlands is mostly done by GPs, but in cases of atypical localized or disseminated disease, patients are often referred to

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medical specialists. According to international guidelines and the recently updated Dutch national guideline, objective clinical findings of early localized LB include erythema migrans (EM), and objective clinical findings of disseminated LB include *Borrelia* lymphocytoma, multiple EM, Lyme arthritis, Lyme carditis and Lyme neuroborreliosis (LNB), among other, more rare manifestations [4–6] (http://www.diliguide.nl/document/1314). Acrodermatitis chronica atrophicans (ACA) is usually referred to as late LB. In case of an EM, which is pathognomonic for LB, no further testing is recommended because EM can precede the antibody response [7,8]. In contrast, serologic testing of antibodies against *B. burgdorferi s.l.* in serum is required to confirm the diagnosis of disseminated LB. When appropriate, the diagnosis of disseminated LB can be further supported by evidence from additional diagnostics, including culture and PCR of *B. burgdorferi s.l.* on skin, synovial fluid or cerebrospinal fluid (CSF) or suggestive histopathologic findings. Although the prognosis of LB after recommended antibiotic treatment is good and microbiological failure appears to be an infrequent event, as discussed elsewhere [4], patients may experience long-lasting and debilitating subjective symptoms despite recommended antibiotic treatment. This condition has been referred to as post-treatment LB syndrome or post–Lyme disease syndrome (PTLBS or PLDS) [4,6], and randomized controlled trials did not show substantial or long-lasting beneficial effects of additional antibiotic treatment compared to placebo [9–12].

With the available serologic tests, it is difficult to differentiate between active and past *B. burgdorferi s.l.* infection, and 4–8% of the Dutch population has detectable antibodies against *B. burgdorferi s.l.* [13]. Therefore, it is not recommended to test patients with subjective symptoms without objective clinical findings compatible with LB. Nonetheless, approximately 70% of the serologic tests ordered by GPs are from such patients [14]. To establish a diagnosis in a patient presenting with subjective symptoms, with a history of tick bites and/or antibodies against *B. burgdorferi s.l.*—previously treated or not treated for LB—can be a challenge for physicians. On the one hand, misdiagnosis of LB can lead to (multiple) antibiotic courses, without effect, but with (serious) side effects or a delay in identification and management of the actual underlying cause of the complaints [15]. On the other hand, when the clinical presentation is less clear or when diagnostic tests are not performed as or when they should be, a missed diagnosis could result in prolonged or progressive illness.

Therefore, in an attempt to offer better care for patients suspected of LB, we have initiated the Amsterdam Multidisciplinary Lyme Borreliosis Center (AMLC). At the AMLC, various medical specialists, including infectious diseases specialists, neurologists, dermatologists and rheumatologists, collaborate to establish a diagnosis in the referred patient—either LB or an alternative diagnosis—and treat accordingly. In this report, we describe the characteristics of the first 200 adult patients who were referred to the AMLC.

Materials and methods

AMLC

The AMLC is located at the outpatient clinic of the Academic Medical Center of the University of Amsterdam in the Netherlands. The AMLC is open to referral of patients by GPs from the Amsterdam region and medical specialist from all over the Netherlands. Referrals were accepted—after being centrally judged by an infectious disease specialist (JH or MvV)—when there was a suspicion of LB, either based on the described symptoms or signs or the results of previous diagnostic tests, or when the referring physician specifically requested referral. On the basis of the provided clinical information, patients were invited to the appropriate outpatient clinic, i.e. the outpatient clinics of infectious diseases, neurology, rheumatology or dermatology. In addition, the department of medical microbiology was frequently consulted. Within each department, there were one or two dedicated specialists who were responsible for patients suspected of having LB. At all AMLC outpatient clinics, information on tick bites, symptoms compatible with LB, previous serologic testing and antibiotic treatment was obtained, and a physical examination in search of objective clinical findings compatible with LB was conducted. In the majority of cases, a *B. burgdorferi s.l.* C6-EIA (IgM/IgG, Immunetics) and upon indication an immunoblot (either IgM and/or IgG) (Mikrogen) was performed by the department of medical microbiology. Tests were considered positive on the basis of the manufacturer's cutoff or interpretation criteria. Patients suspected of having LNB were seen by neurologists. At the department of neurology, lumbar punctures were performed when patients were suspected of having LNB. Patients suspected of having Lyme arthritis were seen by rheumatologists. A synovial fluid aspiration for a *B. burgdorferi s.l.* PCR (Supplementary Information) was performed at the discretion of the treating physician. Patients with skin lesions were seen at the dermatology outpatient clinic, where skin samples were taken for *B. burgdorferi s.l.* culture (Supplementary Information), PCR or histology, upon indication. After this initial (multidisciplinary) evaluation, it was determined whether the patient required (additional) antibiotic treatment for LB. Antibiotic treatment regimes, dosages and duration were in concordance with the recent national guideline from the Dutch institute for healthcare improvement (CBO) (http://www.diliguide.nl/document/1314). Additional testing—such as blood tests and

imaging, to rule out or establish an alternative diagnosis—and therapeutic interventions were left to the discretion of the treating physicians. No systematic follow-up of patients was present at the AMLC. However, follow-up was collected from the documented clinical impression of the treating physician or from the patient's experience if documented. Further follow-up was usually performed through the GP, who was given written advice for further management.

Consecutive retrospective case series and classifications

Case record forms from patients who were referred between January 2011 and April 2013 were retrospectively reviewed using standardized forms. Information on (previsit) diagnostic test results, medical history, objective clinical findings, subjective symptoms and previous treatment was recorded and analyzed by SPSS software, version 21. On the basis of this information, patients were classified into different categories: early localized LB or disseminated LB if patients were not previously treated (Table 1), and persistent *B. burgdorferi s.l.* infection or PTLBS if patients were previously treated (Table 2). To address the likelihood of a causal relationship between complaints and an active or past *B. burgdorferi s.l.* infection, these four categories were further classified as 'definite,' 'probable' and 'questionable.' Definite cases have a low risk, probable cases a low to intermediate risk and questionable cases a high risk of being misclassified. Of note, alternative diagnoses were not found or were considered unlikely in all of the case definitions mentioned above. Finally, patients not fulfilling criteria for any of these categories were classified as not having LB; in some of them, an alternative diagnosis could be found or considered. All cases were reviewed by two reviewers (EH and JC). If there was disagreement between the two reviewers about the classification or if both could not classify the patient into a distinct category, cases were classified by JH. Our retrospective analysis is in accordance with the Academic Medical Center research code, which is based on the Helsinki Declaration of 1975.

Definite early localized LB and disseminated LB. Cases with definite LB included: (a) patients presenting with objective clinical findings compatible with LB as described in national or international guidelines and supportive evidence from laboratory tests, such as *B. burgdorferi s.l.* serologic tests, culture, PCR or suggestive histopathologic findings [4,5] (http://www.diliguide.nl/document/1314); (b) patients with neurologic findings compatible with LNB, as described by the European Federation of Neurological Societies guideline, with a pleocytosis in CSF and positive intrathecal anti–*B. burgdorferi s.l.* IgG antibody index [16] (LNB cases with a duration of symptoms longer than 6 months were considered as late disseminated LB); and (c) patients presenting with objective clinical findings reminiscent of LB, e.g. atypical skin lesions or a polyarthritis without involvement of large joints, which were supported by positive *B. burgdorferi s.l.* culture, PCR and/or suggestive histopathologic findings.

Probable early localized LB and disseminated LB. Cases were classified as probable LB when objective clinical findings reminiscent of LB were present in combination with *B. burgdorferi s.l.* antibodies, or when neurologic findings were compatible with LNB, with only a pleocytosis in CSF or a positive intrathecal anti–*B. burgdorferi s.l.* IgG antibody index, in combination with positive serologic tests for *B. burgdorferi s.l.* antibodies in serum.

Questionable disseminated LB. Cases were classified as questionable disseminated LB when no objective clinical findings compatible with or reminiscent of LB were present in combination with *B. burgdorferi s.l.* antibodies in serum, as determined by serologic tests. In addition, there was either a relation between the onset of symptoms with a tick bite or a non-documented EM or LB manifestation in the past.

Post-treatment LB syndrome. The classification of probable PTLBS was in line with the PLDS criteria from the Infectious Diseases Society of America (IDSA) guidelines [4]. We did not designate this as definite PTLBS because in our opinion, this diagnosis cannot be definite. Cases classified as questionable PTLBS were identical to probable PTLBS cases except that the preceding LB episode was questionable, and cases were required to have positive serologic tests for antibodies against *B. burgdorferi s.l.* We designated these patients as having questionable PTLBS rather than medically unexplained symptoms (MUS) because a relation with a previous *B. burgdorferi s.l.* infection could not be fully excluded.

Persistent B. burgdorferi s.l. infection. Patients presenting with objective clinical findings compatible with LB who had previously been treated with antibiotics were classified as having definite (positive *B. burgdorferi s.l.* culture) or probable (positive *B. burgdorferi s.l.* PCR and/or suggestive histopathologic findings) persistent *B. burgdorferi s.l.* infection. Patients without objective clinical findings but with subjective symptoms that progressed over time despite previous recommended or inappropriate antibiotic treatment for a documented LB episode were classified as having questionable disease. In addition, questionable persistent *B. burgdorferi s.l.* infection included patients presenting with progressive subjective symptoms despite previous inappropriate antibiotic treatment for a questionable LB episode in the past. We considered antibiotic treatment inappropriate when it did not meet guideline recommendations [4,5] (http://www.diliguide.nl/document/1314), i.e. too short a duration, insufficient dosage, insufficient frequency, use of a

TABLE 1. Overview of classification used for diagnosis and probability of LB in patients suspected of LB not previously treated with antibiotics

Probability	Early localized LB	Disseminated LB[a]
Definite	• Typical EM *-or* • Atypical macular skin lesion • Positive *B. burgdorferi s.l.*[c] PCR or culture from skin biopsy	• Objective clinical findings compatible with disseminated LB[b] • Antibodies against *B. burgdorferi s.l.*[c] and/or supportive laboratory evidence[d] *-or* • Objective clinical findings reminiscent of LB[e] • Supportive laboratory evidence[d]
Probable	• Atypical macular skin lesion • History of tick bites • Antibodies against *B. burgdorferi s.l.*[c]	• Neurologic findings suggestive of LNB[f] • Antibodies against *B. burgdorferi s.l.*[c] • Either a pleocytosis in CSF or positive intrathecal IgG AI *-or* • Objective clinical findings reminiscent of LB[e] • Antibodies against *B. burgdorferi s.l.*[c]
Questionable	• NA[i]	• Subjective symptoms only[g] • Antibodies against *B. burgdorferi s.l.*[c] • Nondocumented LB episode in the past[h] or a relation between the onset of symptoms and a tick bite

LB, Lyme borreliosis; EM, erythema migrans; *B. burgdorferi s.l., Borrelia burgdorferi sensu lato;* CSF, cerebrospinal fluid; AI, antibody index; LNB, Lyme neuroborreliosis; NA, not applicable.
Patients not fulfilling these criteria were classified as no LB. Other evident explanations were excluded in patients fulfilling one of these criteria. Definite cases have a low risk, probable cases a low to intermediate risk and questionable cases a high risk of being misclassified. For a more detailed description and explanation, see the Material and Method section.
[a]If duration of symptoms was less than 6 months, cases were classified as early disseminated LB. If duration of symptoms was more than 6 months or it was defined as ACA, which is usually classified as late LB, cases were classified as late disseminated LB.
[b]Based on Stanek *et al.* [5] (http://www.eucalb.com/), which is in line with the guideline from the Dutch institute for healthcare improvement (CBO) (http://www.diliguide.nl/document/1314) and include *Borrelia* lymphocytoma,
multiple EM, Lyme arthritis, Lyme carditis and Lyme neuroborreliosis and ACA.
[c]As determined in serum by a *B. burgdorferi s.l.* C6-EIA (IgM/IgG, Immunetics) and/or immunoblot (either IgM and/or IgG) (Mikrogen).
[d]*B. burgdorferi s.l.* culture or PCR and/or suggestive histopathologic findings.
[e]These include atypical skin lesions, polyarthritis without involvement of large joints, conduction disorders of the heart other than AV-nodal conduction disorders or neurologic symptoms which could be attributed to LB other than a meningoradiculitis, meningoencephalitis or polyradiculitis.
[f]Based on the European Federation of Neurological Societies guidelines by Mygland *et al.* [16].
[g]These include nonspecific symptoms, such as widespread musculoskeletal pain (arthralgia or myalgia), paresthesia or complaints of cognitive impairment with or without fatigue.
[h]LB episode in the past reported by patient, not witnessed by a physician.
[i]With clinical judgment, (repeated) serology and/or skin biopsies for PCR or culture it should be possible to distinguish a probable early localized LB from a non-LB related skin manifestation.

nonrecommended ineffective antibiotic or the simultaneous use of supplements, such as calcium tablets, together with tetracyclines.

Results

Of the patients referred to the AMLC, most were referred by GPs (n = 162, 81%) (Table 3). Fatigue was the most reported complaint, cited by 141 (71%) patients. Other common reported symptoms included arthralgia, myalgia, paresthesia and headache (Table 3). Skin lesions were the most reported objective clinical finding, reported in 31 (16%) patients. More than half of the patients (n = 108, 54%) had symptoms that were present for more than 1 year at the time of presentation at the AMLC; of these, only three had objective clinical findings that were progressive over time. Before referral to the AMLC, for the majority of patients, serologic testing was performed, and approximately half of the patients had received antibiotic treatment based on a suspicion of LB (Table 3).

A *B. burgdorferi s.l.* C6-EIA on serum as part of the AMLC's diagnostic assessment was done in 168 patients (84%) and was considered positive in 66 tested sera (40% of tested sera) (Supplementary Table 1). In the remaining 32 patients, the treating specialist at the AMLC deemed additional testing unnecessary on the basis of a low *a priori* chance of having LB or previous serologic test results (Table 3). For many patients, immunoblot analyses had been performed before referral to the AMLC. Therefore, an immunoblot was performed in only 74 (37%) patients; of these, 28 (38% of tested sera) were positive or indeterminate (Supplementary Table 1). A total of 20 PCRs on skin biopsy samples, synovial fluid and CSF were done to strengthen or confirm the diagnosis of EM, ACA, Lyme arthritis or LNB. In addition, 29 lumbar punctures to detect specific intrathecal antibody production—by C6-EIA—and pleocytosis in CSF were performed to confirm or exclude LNB (Supplementary Table 1). We also tested blood samples of 29 patients—either at the patients' explicit request or because patients had a reported positive PCR blood test from a commercial laboratory before referral—using our clinically validated PCR—and found no positives (data not shown).

A graphical summary of the referral process and the analysis at the AMLC is shown in Fig. 1A. On the basis of the criteria shown in Tables 1 and 2, we concluded that 120 patients (60%)

TABLE 2. Overview of classification used for diagnosis and probability of LB in patients suspected of LB previously treated with antibiotics

Probability	PTLBS	Persistent *B. burgdorferi s.l.* infection
Definite	• NA[a]	• No resolution of previous documented LB episode despite prior antibiotic therapy[b] • Evidence of persistent infection (positive culture)
Probable	• In line with Wormser *et al.*[c] • Previous documented LB episode[d] • Current presentation with only subjective symptoms,[e] despite prior recommended antibiotic therapy[f] • Resolution of symptoms over time	• No resolution of previous documented LB episode despite prior antibiotic therapy[b] • Supportive evidence of persistent infection, i.e. *B. burgdorferi s.l.* PCR and/or suggestive histopathologic findings
Questionable	• Questionable LB episode in the past[g] • Antibodies against *B. burgdorferi s.l.*[h] • Current presentation only with subjective symptoms[e] despite prior recommended antibiotic therapy[f] • Resolution of symptoms over time	• Previous documented LB episode[d] • Persisting subjective symptoms despite antibiotic therapy[b] • No resolution or worsening of symptoms over time • Antibodies against *B. burgdorferi s.l.*[h] *-or* • Questionable LB episode in the past[g] • Prior inappropriate antibiotic therapy[f] • No resolution or worsening of symptoms over time • Antibodies against *B. burgdorferi s.l.*[h]

LB, Lyme borreliosis; PTLBS, Post-treatment LB syndrome; *B. burgdorferi s.l., Borrelia burgdorferi sensu lato.*
Patients not fulfilling these criteria were classified as no LB. Other evident explanations were excluded in patients fulfilling one of these criteria. Definite cases have a low risk, probable cases a low to intermediate risk and questionable cases a high risk of being misclassified. For a more detailed description and explanation, see the Material and Method section.
[a]In our opinion PTLBS cannot be definite.
[b]Either recommended or inappropriate treatment.
[c]For more details, see Wormser *et al.* [4].
[d]Previous objective clinical findings compatible with LB, which were witnessed by a physician and were diagnosed as LB.
[e]These include nonspecific symptoms, such as widespread musculoskeletal pain (arthralgia or myalgia), paresthesia or complaints of cognitive impairment with or without fatigue.
[f]For a description of recommended and inappropriate treatment, see Material and Methods.
[g]LB episode in the past reported by patient, not witnessed by a physician.
[h]As determined in serum by a *B. burgdorferi s.l.* C6-EIA (IgM/IgG, Immunetics) and/or immunoblot (either IgM and/or IgG) (Mikrogen).

did not have LB (Table 4). In 43 of these patients, an alternative diagnosis was established (Supplementary Table 2); for example, seven patients had osteoarthritis. Patients were also diagnosed with human immunodeficiency virus infection, polymyalgia rheumatica or multiple sclerosis, among other diagnoses.

An active form of LB not previously treated with antibiotics was diagnosed in 31 patients (16%), of which only 12 (6%) were classified as definite LB, including five EM, two multiple EM, one Lyme arthritis, one LNB and three ACA (Fig. 1B). In addition, we classified six patients with probable LB, including three patients with skin lesion or lesions—two atypical EM and one atypical multiple EM—and three LNB cases supported by pleocytosis in CSF and *B. burgdorferi s.l.* antibodies in serum (not in CSF). The remaining 13 LB patients were classified as having questionable LB. The most reported symptoms in patients with questionable LB were fatigue, arthralgia, paresthesia, myalgia and headache. In ten patients with questionable LB, a tick bite related to the onset of the symptoms was reported, and in the other three cases, the patients reported a nondocumented and untreated EM in the past.

From the 200 referred patients, 104 had previously received antibiotic treatment. Of these patients, 34 (17%) were diagnosed with PTLBS. We classified 22 patients (11%) as probable PTLBS, meeting the criteria of the published case definition [4], and 12 patients (6%) as having questionable PTLBS (Table 4). Finally, 15 patients (8%) were classified as having persistent *B. burgdorferi s.l.* infection, of which none was classified as definite, three as probable and the majority (n = 12) as questionable. The three patients with probable persistent *B. burgdorferi s.l.* infection included one patient who was diagnosed with a persisting EM—based on ongoing inflammation observed by histopathologic examination of a skin section obtained by skin biopsy—after antibiotic treatment for an EM that had lasted for 2 months. However, *B. burgdorferi s.l.* culture and PCR on skin samples were negative. The second patient was diagnosed with persisting Lyme arthritis after previous antibiotic treatment for Lyme arthritis, supported by a *B. burgdorferi s.l.* PCR on synovial fluid. The third patient presented with recurrent arthritis of the left ankle and IgG antibodies against *B. burgdorferi s.l.* in serum. Before the onset of these symptoms, the patient had been treated for an EM with doxycycline for 10 days, which was followed by a peripheral facial nerve paresis that had resolved over time. These three patients received antibiotic retreatment at the AMLC and clinically improved. In the 12 patients with questionable persistent *B. burgdorferi s.l.* infection, the most reported symptoms were fatigue (n = 8, 67%), arthralgia (n = 7, 58%), paresthesia (n = 6, 50%), headache (n = 6, 50%) and myalgia (n = 4, 33%). In eight of the patients

TABLE 3. Characteristics, presenting symptoms and previsit LB-related diagnostic assessment of 200 patients referred to the Amsterdam Multidisciplinary Lyme Borreliosis Center

Characteristic	Value
Gender	
Male	82 (41%)
Female	118 (59%)
Age, years, median (range)	46 (18–80)
Referred by:	
General practitioner	162 (81%)
Specialist	38 (19%)
Previous referrals to other specialists for current complaints	118 (59%)
Symptoms (top 5)[a]	
Fatigue	141 (71%)
Arthralgia	98 (49%)
Paresthesia	68 (34%)
Myalgia	54 (27%)
Headache	45 (23%)
Duration of symptoms	
<6 weeks	16 (8%)
6 weeks–3 months	20 (10%)
3–6 months	23 (12%)
6–12 months	32 (16%)
More than 1 year	108 (54%)
No symptoms	1 (1%)
Tick bites (time since last tick bite) (n = 200)	
No tick bite	96 (48%)
0–6 months	59 (30%)
6–12 months	5 (3%)
More than 1 year	39 (20%)
Unknown	1 (1%)
Previous *B. burgdorferi s.l.* serology[b]	170 (85%)
Of which positive	127 (75%)
Previous *B. burgdorferi s.l.* PCR	5 (3%)
Of which positive	1 (20%)
Other nonrecommended test[c]	32 (16%)
Of which positive	27 (84%)
Antibiotic treatment	104 (52%)
Doxycycline 100 mg bid <1 month[d]	78 (75%)
Doxycycline 100 mg bid >1 month[d]	14 (13%)
Other antibiotic treatment <1 month[e]	6 (6%)
Other antibiotic treatment >1 month[e]	6 (6%)

Sums of percentages per group may exceed 100% due to rounding.
LB, Lyme borreliosis; *B. burgdorferi s.l., Borrelia burgdorferi sensu lato;* bid, twice a day; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay.
[a]Patients could have reported multiple symptoms.
[b]EIA/ELISA or EIA/ELISA and immunoblot.
[c]Other nonrecommended *B. burgdorferi s.l.* tests include PCR on blood, dark-field microscopy live-blood analysis, lymphocyte transformation test and reduced expression of CD57 on mononuclear cells. Tests were considered positive by the (commercial) laboratory that performed the test.
[d]May be combined with other antibiotic treatment.
[e]May include: amoxicillin, atovaquone, azithromycin, ceftriaxone, ciprofloxacin, larithromycin, metronidazole.

with questionable persistent *B. burgdorferi s.l.* infection, previous antibiotic treatment was regarded as inappropriate because treatment was either too short or because patients had taken calcium or other supplements, which could have lowered absorption of tetracyclines from the intestine. The remaining four patients in the questionable persistent *B. burgdorferi s.l.* infection category reported progressive subjective symptoms after recommended treatment for a documented LB episode, and no alternative explanation was evident.

Antibiotic treatment was provided to 50 patients (25%) by physicians at the AMLC, which included 27 of the 31 patients with early localized or disseminated LB. The remaining four patients had already started with antibiotic therapy, initiated by the referring physician. All patients with probable and questionable persistent *B. burgdorferi s.l.* infection (n = 15) were treated with antibiotics. Finally, eight patients retrospectively classified as having no LB or PTLBS received antibiotic treatment at the AMLC.

Limited information on follow-up—several weeks to months—was available for only 98 patients (49%), making it insufficient for a thorough analysis on follow-up. Nonetheless, we compared the follow-up data from patients with objective clinical findings compatible with or reminiscent of LB—i.e. patients with definite or probable LB and probable persisting *B. burgdorferi s.l.* infection—to that of patients with merely subjective symptoms—i.e. questionable LB and questionable persisting *B. burgdorferi s.l.* infection. In addition, we analyzed the follow-up data of both probable and questionable PTLBS patients. Follow-up data were available from 17 of 21 patients with objective clinical findings compatible with or reminiscent of LB. All of these 17 patients improved. In contrast, in 17 of 25 questionable cases, follow-up data were available, and only eight (47%) reported improvement. In addition, follow-up data were available in 17 of 34 cases with PTLBS—because these were usually referred back to the GP—and 15 (88%) of these 17 patients reported improvement.

Discussion

In this retrospective case series, we classified 200 patients who were referred to our multidisciplinary LB referral clinic. The relatively small number of patients with LB in our study may reflect the societal concerns on LB diagnostics and treatment, the difficulty of excluding LB from the differential diagnosis, a lack of awareness of the current national guidelines by the referring physicians or a lack of power to discriminate between a past and active infection with current serologic tests. In addition, the low number of active *B. burgdorferi s.l.* infections among patients referred to the AMLC could be caused by previous referral to (multiple) other medical specialists, extensive testing on LB and antibiotic treatment before consultation (Table 3). Notably, in 43 (36%) of the 120 AMLC patients who did not have LB, an alternative diagnosis was established (Supplementary Table 2). This illustrates there is a serious risk of improper treatment and misdiagnosis in case of referral or self-referral to 'LB-literate' doctors, who often diagnose these patients with 'chronic Lyme disease' and prescribe prolonged nonrecommended antibiotic treatment [17,18].

A total of 31 patients (16%) were classified as having early localized or disseminated LB. These observations are similar to both reports from the United States [19,20], as well as a recent study from a British LB referral clinic of 115 patients [21], in which 23% of the patients suspected of having LB were thought to have been infected with *B. burgdorferi s.l.* Another study on LNB in Germany reported that of the 113 patients suspected of

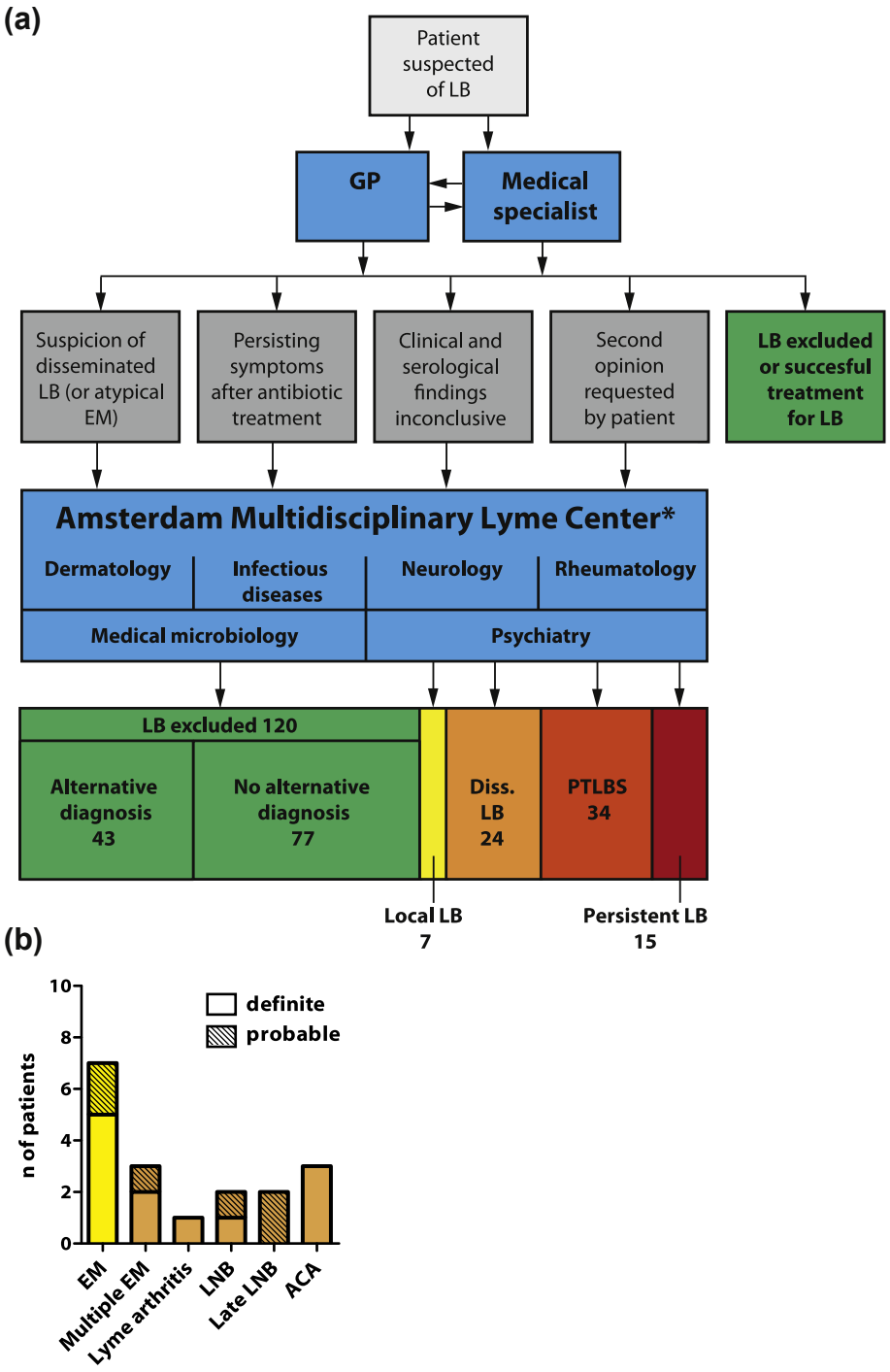

FIG. 1. (a) Graphical summary of 200 patients' referral process to AMLC. *Pediatric infectious diseases are also part of AMLC, but only adults were included in this study. (b) Definite (clear) and probable LB (shaded) cases (n = 18) at AMLC from 200 referred patients. ACA, acrodermatitis chronica atrophicans; Diss. LB, both early and late disseminated LB; EM, erythema migrans; GP, general practitioner; LB, Lyme borreliosis; LNB, Lyme neuroborreliosis; PTLBS, Post-treatment LB syndrome.

chronic LNB, one patient (<1%) had acute LNB, eight patients (7%) had an acute LB—without LNB—and six patients (5%) had residual symptoms after previously proven and treated LNB or LB [22]. Collectively, this illustrates the low number of patients with strong evidence of an active *B. burgdorferi s.l.* infection in LB referral clinics. Indeed, the majority of LB patients in our study (n = 13) were classified as having questionable LB.

TABLE 4. Classification of LB in the 200 patients referred to the Amsterdam multidisciplinary LB center

			Disseminated LB			
	No LB	Early localized LB	<6 months (early)	>6 months (late)	PTLBS	Persistent *B. burgdorferi s.l.* infection
Probability	(*n* = 120)	(*n* = 7)	(*n* = 7)	(*n* = 17)	(*n* = 34)	(*n* = 15)
Definite	120 (100%)	5 (71%)	4 (57%)[a]	3 (18%)[b]	NA[c]	0 (0%)
Probable	NA	2 (29%)	2 (29%)[d]	2 (12%)[e]	22 (65%)	3 (20%)[f]
Questionable	NA	NA	1 (14%)	12 (70%)	12 (35%)	12 (80%)

Classification is based on the criteria and definitions shown in Tables 1 and 2. For LNB criteria, see Material and Methods.
LB, Lyme borreliosis; *B. burgdorferi s.l., Borrelia burgdorferi sensu lato;* PTLBS, post-treatment LB syndrome; NA, not applicable; LNB, Lyme neuroborreliosis; MEM, multiple erythema migrans; CSF, cerebrospinal fluid.
[a]One LNB, one Lyme arthritis and two MEM.
[b]Three ACA.
[c]See Tables 1 and 2.
[d]One LNB, with pleocytosis but without *B. burgdorferi s.l.* antibody production in CSF, and one MEM.
[e]Two late LNB, with pleocytosis but without *B. burgdorferi s.l.* antibody production in CSF.
[f]Cases are described in text.

Patients classified as having questionable LB presented with subjective symptoms only. In general, these subjective symptoms have no predictive value for LB. However, patients with questionable LB in our study had positive *B. burgdorferi s.l.* serologic tests in combination with either a relation between the onset of symptoms with a tick bite or a nondocumented EM or LB manifestation in the past. In addition, after careful examination by multiple specialists at the AMLC, another explanation could not be demonstrated. It could be debated whether instead of questionable LB we could have designated these patients as having possible, improbable or dubious LB. Regardless, on the basis of the CBO guideline, which recommends treating cases with a low *a priori* chance for LB and positive *B. burgdorferi s.l.* serologic tests, we chose to treat these patients with antibiotics. We do not recommend that GPs and physicians outside LB referral clinics follow our approach, and we emphasize that careful exclusion of other causes and consultation of other specialists is of paramount importance for this patient category. We discussed with these patients that if the recommended antibiotic therapy had no long-lasting effects, LB was unlikely to be the cause of their symptoms. The benefit of our approach might be that both physician and patient can focus on additional investigations in search of the etiology or—perhaps more often—adequate management of MUS when antibiotics did not have any effects. On the contrary, when symptoms did resolve, a positive response to antibiotics does not necessarily mean the patient was infected with *B. burgdorferi s.l.* because a placebo effect, an immunomodulatory effect of the antibiotic or the mere effect of time could be alternative explanations.

Another 34 (17%) of the referred patients were diagnosed with PTLBS, of whom 12 were classified as questionable PTLBS, since a relation with preceding *B. burgdorferi s.l.* infection could not be ignored because of *B. burgdorferi s.l.* serology and no evident alternative cause. However, an active infection with *B. burgdorferi s.l.* was considered highly unlikely. The symptoms of the PTLBS patients are nonspecific and share similarities with those of chronic fatigue syndrome, fibromyalgia or MUS. The treatment for patients diagnosed with PTLBS and MUS is similar and does not include antibiotic treatment, but an individualized approach is necessary to achieve acceptance and improvement of quality of life in which the GPs plays a central role. Specialized MUS centers can provide multidisciplinary education and advice or even cognitive therapy. Indeed, the AMLC internally referred four patients to a psychiatrist with expertise in the management of MUS. Recently, after the completion of this study, the AMLC begun a collaboration with a MUS center at the VU University Medical Center Amsterdam.

Finally, of the 15 cases of persistent *B. burgdorferi s.l.* infection, none was classified as definite and three were classified as probable. The absence of definite persistent *B. burgdorferi s.l.* infection cases and the low number of probable persistent *B. burgdorferi s.l.* infection cases in our study is not unexpected because a persistent *B. burgdorferi s.l.* infection after recommended antibiotic treatment appears to be rare [4,23]. It is also possible that complaints, if caused by an active *B. burgdorferi s.l.* infection, are the result of a reinfection rather than a persistent infection [24]. The 12 patients diagnosed with questionable persistent *B. burgdorferi s.l.* infection had subjective symptoms only, similar to patients diagnosed with questionable LB, with the difference that they had received (inappropriate) antibiotic treatment for a prior (questionable) LB episode and that their symptoms were progressive over time (Table 2). Although partially against published trials [9–12], and not recommended by the IDSA guidelines [4], retreatment of questionable persistent *B. burgdorferi s.l.* infection cases, especially those in patients who had had received prior inappropriate treatment, is in accordance with recommendations from the recent Dutch national guidelines (http://www.diliguide.nl/document/1314) and was the result of a compromise between physician and patient.

As we did with questionable LB patients, we discussed the pros and cons of antibiotic treatment in patients with questionable persistent *B. burgdorferi s.l.* infection. Specifically, we discussed the fact that if their LB did not respond to antibiotic therapy, a persistent *B. burgdorferi s.l.* infection was unlikely, and we discussed the option of treatment for MUS. Our classification 'questionable persistent *B. burgdorferi s.l.* infection' might be useful to describe the patient population in a (tertiary) Lyme clinic. However, because the risk of misclassifying these patients is high, such cases should only be used with caution for future clinical or research purposes. Furthermore, this classification should not be confused with 'chronic Lyme disease,' which is a misnomer describing patients with chronic subjective symptoms that are attributed to LB but that is in fact a heterogenous group, as previously described [16,25].

Although incomplete and limited, our follow-up analysis showed that antibiotic treatment resulted more often in improvement in patients with objective clinical findings compatible with or reminiscent of LB compared to patients in whom only subjective symptoms were present. In future studies, we will strive for more accurate and complete follow-up over a longer period of time, which will be facilitated in the near future by a multicenter prospective study assessing the risk of, and the risk factors for, developing persisting symptoms after treated LB. In addition, once the number of well-defined (definite and probable) LB cases increases, we will perform multiple logistic regression analysis to identify negative and/or positive predictors for LB.

To conclude, LB is an infectious disease to which specific objective clinical findings have been attributed. However, LB is invariably linked by many to a wide range of subjective symptoms, limited diagnostic test options and poor treatment options and outcomes. This affects the use of diagnostic tests for and treatment of LB by physicians. In the current study, we used established criteria and also proposed new criteria to categorize patient populations at LB referral centers. Using these criteria, we show that we were able to exclude LB in many cases, to establish alternative diagnoses for a significant group of patients and to categorize most of the patients into distinct classifications. Using the currently available diagnostic tests, for some patients—especially questionable LB and questionable persisting *B. burgdorferi s.l.* infection cases—it is difficult to determine whether these patients indeed had a symptomatic *B. burgdorferi s.l.* infection. Future tests might be able to better distinguish between past and active *B. burgdorferi s.l.* infections and could thus partially resolve these issues and guide antibiotic treatment. Until these tests are developed, validated and widely available, physicians with both experience with and affinity for LB should determine the likelihood of an active infection with *B. burgdorferi s.l.* in each individual patient. The benefits of a tertiary referral center for LB—such as the AMLC—are that this evaluation is done in a multidisciplinary and systematic manner by experienced specialists, it can initiate and engage in basic and clinical research on LB and it will uncover alternative diagnoses. Thus, tertiary LB referral centers are in direct interest of LB (suspected) patients.

Transparency declaration

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.11.014.

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