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Original article

Classification of patients referred under suspicion of tick-borne diseases, Copenhagen, Denmark

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ABSTRACT

To provide better care for patients suspected of having a tick-transmitted infection, the Clinic for Tick-borne Diseases at Rigshospitalet, Copenhagen, Denmark was established. The aim of this prospective cohort study was to evaluate diagnostic outcome and to characterize demographics and clinical presentations of patients referred between the 1st of September 2017 to 31st of August 2019.

A diagnosis of Lyme borreliosis was based on medical history, symptoms, serology and cerebrospinal fluid analysis. The patients were classified as definite Lyme borreliosis, possible Lyme borreliosis or post-treatment Lyme disease syndrome. Antibiotic treatment of Lyme borreliosis manifestations was initiated in accordance with the national guidelines. Patients not fulfilling the criteria of Lyme borreliosis were further investigated and discussed with an interdisciplinary team consisting of specialists from relevant specialties, according to individual clinical presentation and symptoms. Clinical information and demographics were registered and managed in a database.

A total of 215 patients were included in the study period. Median age was 51 years (range 17–83 years), and 56 % were female. Definite Lyme borreliosis was diagnosed in 45 patients, of which 20 patients had erythema migrans, 14 patients had definite Lyme neuroborreliosis, six had acrodermatitis chronica atrophicans, four had multiple erythema migrans and one had Lyme carditis. Furthermore, 12 patients were classified as possible Lyme borreliosis and 12 patients as post-treatment Lyme disease syndrome. A total of 146 patients (68 %) did not fulfil the diagnostic criteria of Lyme borreliosis. Half of these patients (73 patients, 34 %) were diagnosed with an alternative diagnosis including inflammatory diseases, cancer diseases and two patients with a tick-associated disease other than Lyme borreliosis. A total of 73 patients (34 %) were discharged without sign of somatic disease. Lyme borreliosis patients had a shorter duration of symptoms prior to the first hospital encounter compared to patients discharged without a specific diagnosis ($p < 0.001$). When comparing symptoms at presentation, patients discharged without a specific diagnosis suffered more often from general fatigue and cognitive dysfunction.

In conclusion, 66 % of all referred patients were given a specific diagnosis after ended outpatient course. A total of 32 % was diagnosed with either definite Lyme borreliosis, possible Lyme borreliosis or post-treatment Lyme disease syndrome; 34 % was diagnosed with a non-tick-associated diagnosis. Our findings underscore the complexity in diagnosing Lyme borreliosis and the importance of ruling out other diseases through careful examination.

1. Introduction

Lyme borreliosis, a tick-borne infection caused by the spirochetes of the *Borrelia burgdorferi* sensu lato complex (*B. burgdorferi* sl), is the most

prevalent vector-borne infection in Europe. The annual incidence in Europe varies from 1/100,000 to more than 100/100,000 inhabitants in different countries (Sykes and Makiello, 2017; Rizzoli et al., 2011; van den Wijngaard et al., 2017; Stanek et al., 2011). Due to climatic and

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environmental changes, leading to a longer tick activity period, the number of Lyme borreliosis cases are expected to increase in the future (Lindgren et al., 2000; Kullberg et al., 2020; Petrulioniene et al., 2020).

Since the discovery of *B. burgdorferi* s.l, a wide entity of criteria to diagnose Lyme borreliosis has been used. However, during the last 25 years standardized and evidence-based European and American guidelines for diagnosis and treatment of early and disseminated manifestations of Lyme borreliosis have been established (Eldin et al., 2019; Kullberg et al., 2020; Mygland et al., 2010). The most common clinical manifestation of Lyme borreliosis is erythema migrans, but *B. burgdorferi* s.l can disseminate, causing Lyme neuroborreliosis, Lyme arthritis, Lyme carditis or acrodermatitis chronica atrophicans (Eldin et al., 2019). Despite the general public awareness of Lyme borreliosis, and the fact that Lyme neuroborreliosis has been included in the European epidemiological surveillance since 2018, a significant treatment delay is often reported (Knudtzen et al., 2017; Ogrinc et al., 2016).

Although, the prognosis of Lyme borreliosis after recommended antibiotic therapy is good, some patients experience long-standing and even chronic non-specific symptoms such as fatigue, cognitive difficulties, headache, and musculoskeletal pain. If those symptoms are present more than 6 months, the condition is referred to as post-treatment Lyme disease syndrome (Mygland et al., 2010; Coumou et al., 2015; Wormser et al., 2006; Kullberg et al., 2020). Much speculation exists concerning the syndrome, but no study has yet been able to demonstrate a direct cause. However, current literature does not support post-treatment Lyme disease syndrome as a persistent *B. burgdorferi* s.l infection and any long-lasting benefits from additional antibiotics is not documented (Koedel et al., 2015; Nemeth et al., 2016).

In general, the commonly used tests for *B. burgdorferi* s.l antibodies have a high analytical sensitivity and specificity (Leefflang et al., 2016). However, there are some limitations regarding serology. It takes time for the immune system to initiate antibody production upon infection, and antibodies persist for a long time after an infection. The limited understanding of these limitations and the widespread use of serology causes an unsatisfying diagnostic performance and may lead to considerable overdiagnosis (Raffetin et al., 2020; Webber et al., 2019).

Throughout Europe awareness concerning Lyme borreliosis has increased and as a result of the complex symptomatology and many possible alternative diagnoses, more countries have set up multidisciplinary centers for patients with Lyme borreliosis suspicion (Coumou et al., 2015; Jacquet et al., 2019; Cottle et al., 2012; Zomer et al., 2017). Moreover, a considerable proportion of patients suspected of Lyme borreliosis are reported to be diagnosed with rheumatological, neurological or other diseases underlining the need of careful medical examination (Jacquet et al., 2019). Thus, patients referred under suspicion of Lyme borreliosis, presumed Lyme borreliosis or post-treatment Lyme disease syndrome should be evaluated appropriately in order to establish a diagnosis of Lyme borreliosis or to rule out ongoing disease.

The aim of the present prospective study was to evaluate the diagnostic outcome and to describe the clinical characteristics of the first 215 adult patients referred to the Clinic for Tick-borne Diseases, Copenhagen, Denmark.

2. Material and methods

We performed a prospective observational cohort study at the Clinic for Tick-borne Diseases as part of the Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark. In 2017, we established the Clinic for Tick-borne Diseases to improve management of patients suspected of Lyme borreliosis. A multi-disciplinary network of various medical specialists collaborated to establish a diagnosis in referred patients.

2.1. Setting and study population

We included adult patients living in the eastern part of Denmark,

referred from general practitioners, other medical specialists and hospital departments, under suspicion of Lyme borreliosis. The study was conducted from 1st of September 2017 to 31st of August 2019. Children suspected of Lyme borreliosis were seen by pediatricians.

All patients were seen by the same specialist in infectious diseases. On the first visit, a thorough medical history was obtained and any former diagnostic tests were evaluated, including laboratory tests and imaging modalities such as x-ray; magnetic resonance imaging (MRI), computer tomography (CT) or positron-emission tomography in combination with CT (PET/CT) and previous antimicrobial treatment. A physical examination was performed, and basic laboratory tests including hemoglobin, white blood cells differential count, liver and kidney function test, C-reactive protein (CRP) and thyroid function test was taken. If relevant, additional specific blood testing was performed including Immunoglobulin (Ig)G, IgA, IgM, Peptidyl-Dipeptidase A (ACE), antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), anti-cyclic citrullinated peptide (anti-CCP), vitamin D, ferritin, sedimentation rate and M-component.

Serum samples from all patients were analyzed for serum *B. burgdorferi* s.l antibodies in addition with antibodies for tick-borne encephalitis (TBE), *Anaplasma phagocytophilum*, *Rickettsia* spp., *Bartonella henselae*, *Francisella tularensis* and *Babesia* spp., if clinical relevant. Additional imaging was performed during the outpatient course when appropriate.

All patients suspected of Lyme neuroborreliosis had a lumbar puncture performed and cerebrospinal fluid (CSF) was examined for CSF-leucocytes, protein, lactate, glucose ratio, chemokine ligand 13 (CXCL13) and intrathecal *B. burgdorferi* s.l antibody production. Patients suspected of having Lyme arthritis, connective tissue diseases or other rheumatological diseases were assessed by the same rheumatologist. Patients suspected of non-infectious neurological diseases, were assessed by a neurologist and finally patients suspected of non-infectious dermatological conditions were referred to a dermatologist. Antibiotic treatment of Lyme borreliosis manifestations was initiated in accordance with the national guidelines (Dessau et al., 2014). Patients with confirmed disseminated Lyme borreliosis had a follow-up one, three and six months after treatment initiation. Some patients required additional follow-up, which was accommodated.

If an alternative diagnosis was suspected during the diagnostic work-up, further investigations were initiated, and the patients were referred to the relevant specialty. Patients assessed in the Clinic for Tick-borne Diseases, having severe functional limitations and physical symptoms, but where medical reasons had been excluded, were referred to the recently established Center for Complex symptoms at Frederiksberg Hospital. The center evaluates patients with unexplained complex symptoms and facilitates individual multidisciplinary treatment courses in collaboration between psychologists, physiotherapists, social- and medical professionals.

2.2. Patient data

We collected information regarding baseline demographics, medical history, the diagnostic work-up and final diagnoses. Medical history included referral specifications, history and time of tick bite and erythema migrans, duration and specification of symptoms and antibiotic treatment. If the patient had an ongoing outpatient course at a private clinic or abroad, this was registered as well as number of referred patients with self-paid results of non-validated tests of *B. burgdorferi* s.l antibodies. Duration of symptoms was defined as from debut of symptoms to the first visit at the Clinic for Tick-borne Diseases. If the symptoms resolved before assessment, the symptoms duration would be by the time the symptoms resolved. The symptoms duration was categorized in 5 subgroups; ≤ 6 weeks, > 6 weeks to ≤ 3 months, > 3 months to ≤ 6 months, > 6 months to ≤ 12 months and > 1 year. Following symptoms were registered; general fatigue, cranial nerve palsy, motor and sensory nerve palsy, headache, cognitive dysfunction (self-

reported), weight change, sleep disturbances, radiating pain, myalgia, dizziness, arthralgia and fever.

The final diagnoses were categorized as either definite early localized Lyme borreliosis, definite disseminated Lyme borreliosis, possible disseminated Lyme borreliosis, post-treatment Lyme disease syndrome, no specific diagnosis or an alternative specific diagnosis (Mygland et al., 2010; Wormser et al., 2006; Kullberg et al., 2020).

2.3. Outcomes

Primary outcomes of this study were final diagnoses defined as definite Lyme borreliosis, number of non-tick-associated diagnoses and number of patients without a specific diagnosis after ended outpatient course. Secondary outcomes were clinical characteristics and diagnostic work-up.

2.4. Definitions

The definitions of clinical Lyme borreliosis manifestations were performed in line with published European guidelines and case definitions (Stanek and Strle, 2018; Stanek et al., 2011; Mygland et al., 2010; Eldin et al., 2019; Kullberg et al., 2020; Dessau et al., 2014).

2.4.1. Erythema migrans

We defined erythema migrans as early localized Lyme borreliosis, solely from the clinical presentation (Eldin et al., 2019; Kullberg et al., 2020; Dessau et al., 2014).

2.4.2. Multiple erythema migrans

We defined multiple erythema migrans as early disseminated infection solely from the clinical presentation (Eldin et al., 2019).

2.4.3. Lyme neuroborreliosis

In line with EFNS guidelines on the diagnosis of European Lyme neuroborreliosis, three criteria should be fulfilled for definite Lyme neuroborreliosis: (i) neurological symptoms suggestive of Lyme neuroborreliosis with other causes excluded; (ii) cerebrospinal fluid pleocytosis; (iii) specific *B. burgdorferi* sI antibodies in CSF, intrathecally produced (Mygland et al., 2010). Possible Lyme neuroborreliosis was diagnosed in patients either fulfilling two of three of the criteria above (Mygland et al., 2010) or fulfilling the following; (i) CSF investigations were not performed due to contraindications to lumbar puncture; (ii) presentation with typical clinical features consistent with Lyme neuroborreliosis; (iii) presence of specific serum *B. burgdorferi* sI IgG or IgM antibodies; (iiii) exclusion of other possible causes (Rauer et al., 2018).

2.4.4. Post-treatment Lyme disease syndrome

If symptoms, related to a previously verified and treated Lyme borreliosis manifestation, persisted for more than 6 months, the condition was termed post-treatment Lyme disease syndrome (Mygland et al., 2010; Coumou et al., 2015; Wormser et al., 2006; Kullberg et al., 2020).

2.4.5. Lyme carditis

Lyme carditis was diagnosed in patients presenting with acute onset of varying degree of intermittent atrioventricular (A–V) heart block, where other causes of cardiac disease were ruled out, and where specific serum *B. burgdorferi* sI IgG and/or IgM antibodies were present (Stanek et al., 2011; Eldin et al., 2019).

2.4.6. Lyme arthritis

The diagnosis of Lyme arthritis was based on the medical history, clinical features, exclusion of other causes of arthritis and the presence of *B. burgdorferi* sI IgG antibodies in serum. PCR detection of *B. burgdorferi* sI DNA in synovial fluid was attempted if possible (Stanek and Strle, 2018; Eldin et al., 2019).

2.4.7. Acrodermatitis chronica atrophicans

Acrodermatitis chronica atrophicans was diagnosed, if the clinically presentation was compatible with acrodermatitis chronica atrophicans and high level of serum *B. burgdorferi* sI IgG antibodies were detected. If possible, a skin sample was obtained for histological examination to further confirm the diagnosis (Eldin et al., 2019).

2.5. Laboratory testing

For the detection of serum *B. burgdorferi* sI antibodies the IDEIA *B. burgdorferi* sI IgG (Oxoid Hampshire, United Kingdom) was used. The IDEIA flagella antigen-based enzyme-linked immunosorbent assay (ELISA) Lyme neuroborreliosis test (Oxoid Hampshire, United Kingdom) was used for detection of intrathecal synthesis of *B. burgdorferi* sI specific IgG and IgM antibodies (Hansen and Lebech, 1991). An antibody production index > 0.3 was considered as positive according to the manufacturer's instructions. For specification of assays and antibody cut-off values for *Rickettsia* spp., *A. phagocytophilum*, *B. henselae*, *Babesia* spp. and TBE see (Ocias et al., 2020). *F. tularensis* antibodies were tested by an in-house agglutination test.

2.6. Statistical analysis

Categorical variables are reported as proportions and percentages. Comparison between groups were tested by Chi-square or Fischer's exact test as appropriate. Comparison of age distribution and symptoms duration between groups was tested by Mann-Whitney U test. A *p*-value < 0.05 was used for statistical significance. All analyses were performed using SAS Enterprise Guide, version 7.1.

3. Results

In total, 223 adult patients were evaluated in the Clinic for Tick-borne Diseases from 1st of September 2017 to 31st of August 2019. In total, 8 patients were excluded due to either invalid registration (*n* = 5) or doublet registration (*n* = 3) (Fig. 1).

Median age was 51 years (range 17–83) and 56 % were female. Most patients were referred by their general practitioner (58 %). History of a verified tick bite and erythema migrans was registered in 149 (69 %) and 81 patients (38 %), respectively. Of the patients recalling a previous tick bite, 41 % reported a verified tick bite >1 month to ≤ 6 months prior to referral. Clinical characteristics of the referred patients are outlined in Table 1.

At the time of the first visit, 144 patients (53 %) were seropositive with either IgG or IgM *B. burgdorferi* sI antibodies. Of the patients, diagnosed with a non-tick-associated disease, 50 % had detectable serum *B. burgdorferi* sI IgG or IgM antibodies. Serum *B. burgdorferi* sI was not measured in 16 patients (7%). They either presented with erythema migrans, did not have symptoms consistent with Lyme borreliosis, or presented with acute neurological symptoms and had a lumbar puncture performed immediately.

Of the patients diagnosed with definite Lyme borreliosis, 58 % recalled previous erythema migrans. In total, 37 % of the patients discharged without a specific diagnosis recalled previous erythema migrans.

A lumbar puncture was performed in 93 patients (43 %); 28 patients had the lumbar puncture performed prior to referral and 5 patients had both a lumbar puncture performed prior to and at referral. Overall, 59 % of the patients were already treated for Lyme borreliosis according to national guidelines, prior to referral.

In total, 18 patients (8%) had under suspicion of tick-borne diseases consulted private clinics broad (e.g. Germany and Poland) and 19 patients (9%) a private clinic in Denmark, not covered by the Danish health insurance. Whereas, 6 patients had consulted both a private clinic in Denmark and abroad. Of the 31 patients treated abroad or at a private clinic, 30 patients did not fulfil the definitions of clinical Lyme

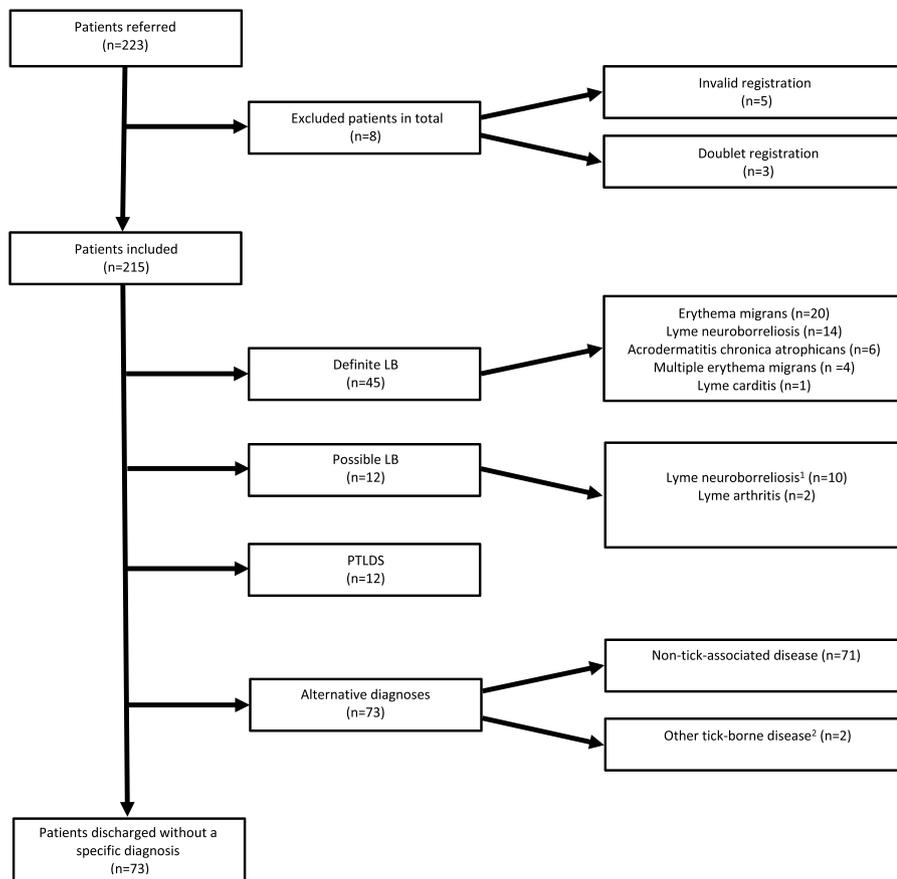


Fig. 1. Patients referred to the Clinic for Tick-borne diseases, 2017-2019, Rigshospitalet, Copenhagen, Denmark.

Abbreviations: LB, Lyme borreliosis; LP, lumbar puncture; PTLDS, post-treatment Lyme disease syndrome; CSF, cerebrospinal fluid.

¹In 6 patients the diagnosis was based on clinical symptoms, positive serum *B.burgdorferi* antibodies and clinical response to antibiotics. CSF analysis was not performed due to contraindications for lumbar puncture.

² Diagnosed with rickettsiosis.

borreliosis manifestations according to European guidelines and case definitions (Stanek and Strle, 2018; Stanek et al., 2011; Mygland et al., 2010; Eldin et al., 2019; Kullberg et al., 2020; Dessau et al., 2014).

3.1. Definite Lyme borreliosis

In total, 21 % of referred patients were diagnosed with definite Lyme borreliosis; definite early localized Lyme borreliosis was present in 20 patients (9%) and 25 patients (12 %) were diagnosed with definite disseminated Lyme borreliosis.

Of the patients with disseminated Lyme borreliosis:

- Fourteen patients fulfilled the criteria for definite Lyme neuroborreliosis.
- Six patients were diagnosed with acrodermatitis chronica atrophicans.
- Four patients were diagnosed with multiple erythema migrans.
- One was diagnosed with Lyme carditis.

3.2. Possible disseminated Lyme borreliosis

In total, 6% of referred patients were diagnosed with possible disseminated Lyme borreliosis.

- Six patients had typical clinical symptoms consistent with Lyme neuroborreliosis and presence of serum *B. burgdorferi* sI IgG antibodies. Due to contraindications for lumbar puncture, no CSF investigation was performed.

They were treated with oral doxycycline for 14 days and remission of symptoms were seen in all cases.

- Four patients fulfilled two diagnostic criteria of Lyme neuroborreliosis and were diagnosed as possible Lyme neuroborreliosis.
- Two patients were categorized as possible Lyme arthritis. Both patients presented with monoarthritis (one elbow, one knee) and high levels of *B. burgdorferi* sI IgG antibodies in serum were detected. Synovial fluid for definite diagnosis was not possible to obtain in the acute phase. Other medical conditions were out ruled in both cases by a rheumatologist. Both patients were treated with oral doxycycline for 21 days.

3.3. Post-treatment Lyme disease syndrome

In total, 6% of referred patients presented with persisting symptoms for more than 6 months and were classified with post-treatment Lyme disease syndrome. All had a history of verified and treated Lyme neuroborreliosis. To out rule active Lyme neuroborreliosis, nine patients had a post treatment lumbar puncture performed. The CSF investigations showed no sign of active infection. In three patients, CSF investigation was not performed due to contraindications to lumbar puncture.

3.4. Not all patients were discharged with a diagnosis

In total, 146 patients did not have Lyme borreliosis. Of these, 73 patients (34 %) did not get a specific diagnosis after ended outpatient course. Physical examination and paraclinical investigations in these patients were normal. The patients were reassured, handed information on how to handle future tick bites and to contact their general practitioner when suspicious of erythema migrans.

Table 1

Baseline characteristics of 215 adults at the time of referral to the Clinic for Tick-borne Diseases 2017-2019, Rigshospitalet, Copenhagen, Denmark.

Characteristics	No. of patients (%) or median (IQR)
Age, years, median (IQR)	51 (39–63)
Gender female	121 (56)
Referred by	
General practitioner	124 (58)
Hospital department	64 (30)
Specialist	16 (7)
Private Hospital	3 (1)
Other	8 (4)
History of tick bite	149 (69)
Last verified tick bite	
≤ 1 month ago	16 (11)
> 1 month to ≤ 6 months ago	61 (41)
> 6 months to ≤ 1 year ago	22 (15)
> 1 year	35 (24)
Not recorded	15 (10)
History of EM	81 (38)
Duration of symptoms	
≤ 6 weeks	62 (29)
> 6 weeks to ≤ 3 months	39 (18)
> 3 months to ≤ 6 months	36 (17)
> 6 months to ≤ 12 months	19 (9)
> 1 year	58 (28)
Prior antibiotic treatment	126 (59)
Laboratory results prior to or at referral	
Serum <i>B. burgdorferi</i> sI IgG or IgM positive	114 (53)
Serum <i>B. burgdorferi</i> sI IgG and IgM negative	69 (32)
No measurement of serum <i>B. burgdorferi</i> sI antibodies performed	16 (7)
CSF investigation	93 (43)
<i>B. burgdorferi</i> sI AI IgG or IgM positive	25 (27)
<i>B. burgdorferi</i> sI AI IgG and IgM negative	68 (73)
CSF leucocytes (> 5 × 10 ⁶ cells/L)	28 (29)

Abbreviations: EM, erythema migrans; IQR, Interquartile Range; LNB, Lyme neuroborreliosis; AI, antibody production index; *B. burgdorferi* sI, *Borrelia burgdorferi* sensu lato complex; CSF, cerebrospinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M.

3.5. Neurological diseases were most frequent among patients with a non-tick-associated diagnosis

A non-tick-associated manifestation after ended outpatient course

was diagnosed in 71 patients (33 %) and two patients (1%) were diagnosed with *Rickettsiosis*. Diagnoses are listed in Table 2. Most prevalent non-tick-associated conditions were rheumatological diseases and neurological diseases. Of the rheumatological diseases, polymyalgia

Table 2

Non-tick-associated diagnoses established at the end of the outpatient course as part of the multidisciplinary management of 215 patients referred to the Clinic for Tick-borne Diseases 2017-2019, Rigshospitalet, Copenhagen, Denmark.

Rheumatological diseases (n=13)	Neurological diseases (n=17)	Dermatological and venereological diseases (n=5)	Verified or suspected viral or bacterial infection (n=13)	Oto- rhino- pharyngeal diseases (n=2)	Endocrine diseases (n=3)	Other (n=14)
Polymyalgia rheumatica (4)	Discus degeneration/ spinal stenosis (7)	Erythema annulare centrifugum (1)	Suspected viral infection, spontaneous recovery (3)	Tumor in cavum nasi (1)	Hyper-/ hypothyroidism (2)	Side effect to medication ¹ (3)
Reactive arthritis (3)	Comotio cerebri/ chronic headache (3)	Herpes genitalia (1)	Acute Cytomegalovirus infection (2)	Thyroid tumor (1)	Mb. Addison (1)	Chronic pain syndrome/ fatigue syndrome (3)
Rheumatoid arthritis (1)	Bell's palsy (2)	Psoriasis (1)	Sinusitis (2)			Carpal tunnel syndrome (2)
Spondyloarthritis (1)	Neurasthenia (1)	Other dermatological rash (2)	Suspected Bartonella (2)			MGUS (1)
Psoriasis arthritis (1)	Trigeminal neuralgia (1)		Sequela due to gastroenteritis (2)			Seminoma, possible sequelae due to operation (1)
Fibromyalgia (1)	Suspected polyneuropathy (1)		Pneumonia (1)			Oligodendroglioma, possible sequelae due to operation (1)
Sarcoidosis (1)	Plexus brachialis lesion (1)		Viral meningitis (1)			M. brevis tendon degeneration (1)
Other (1)	Arteria spinalis anterior occlusion (1)					Elevated urate, dialysis patient (1)
						Prostate malignancy (1)

Abbreviations: Mb., morbus; MGUS, monoclonal gammopathy of undetermined significance; M., musculus.

¹ Tamoxifen, docetaxel, sertraline.

Table 3

Comparison between patients diagnosed with definite Lyme borreliosis and patients discharged without a specific diagnosis at the Clinic for Tick-borne Diseases 2017–2019, Rigshospitalet, Copenhagen, Denmark.

	Definite Lyme borreliosis (n = 45)	No specific diagnosis ¹ (n = 73)	P-value ²
Age, median (IQR)	57 (40–65)	48 (38–57)	0.06
Gender, female, n (%)	26 (58)	41 (56)	0.9
History of tick-bite, n (%)	29 (64)	54 (74)	0.3
History of EM, n (%)	26 (58)	27 (37)	0.3
Duration of symptoms, n (%)			
≤ 6 weeks	22 (50)	17 (23)	
> 6 weeks to ≤ 3 months	12 (27)	9 (12)	
> 3 months to ≤ 6 months	5 (11)	17 (23)	< 0.0001
> 6 months to ≤ 12 months	3 (7)	7 (10)	
> 1 year	2 (5)	23 (32)	
Missing	1	0	
Symptoms, n (%)			
General fatigue	11 (32)	38 (60)	0.001
Missing	11	10	
Headache	11 (28)	27 (39)	0.2
Missing	5	4	
Cognitive dysfunction	3 (8)	16 (23)	0.04
Missing	5	3	
Dizziness	6 (15)	16 (26)	0.2
Missing	6	12	
Weight change	3 (8)	13 (22)	0.08
Missing	9	15	
Sleep disturbance	7 (20)	8 (15)	0.5
Missing	10	19	
Fever	8 (20)	11 (17)	0.7
Missing	4	7	
Arthralgia	11 (24)	26 (37)	0.2
Missing	0	2	
Myalgia	18 (41)	38 (53)	0.2
Missing	1	1	
Cranial nerve palsy	5 (11)	6 (8)	0.2
Missing	0	1	
Sensory nerve palsy	10 (23)	19 (26)	0.7
Missing	2	1	
Motor nerve palsy	7 (15)	2 (3)	0.01
Missing	1	1	
Radiating pain	18 (43)	18 (25)	0.05
Missing	3	2	

Abbreviations: IQR, Interquartile range; EM, erythema migrans.

¹ Includes patients who did not get a specific diagnosis after ended out-patient course.

² Comparison between groups were tested by Chi-square test or Fischer's exact test as appropriate. Age distribution and symptoms duration distribution between groups were tested by Mann-Whitney U test.

rheumatica (n = 4) and reactive arthritis (n = 3) were most common and of the neurological diseases, disc degeneration/spinal stenosis (n = 7), commotio cerebri/chronic headache (n = 3) and Bell's palsy (n = 2) were most prevalent. Three patients were diagnosed with cancer.

3.6. Patients discharged without a specific diagnosis presented more often with long symptoms duration and cognitive dysfunction

When comparing Lyme borreliosis patients to patients discharged without specific diagnoses, no significant differences were seen in the distribution of age, gender, history of tick-bite or erythema migrans (*p*-values are listed in Table 3).

Patients without a specific diagnosis were more likely to have a longer duration of symptoms compared to Lyme borreliosis patients (*p* < 0.0001); 50 % of the Lyme borreliosis patients had symptoms for less than 6 weeks compared with 23 % of the patients discharged without a specific diagnoses, whereas 32 % of the patients discharged without specific diagnosis had symptoms for more than one year prior to assessment compared with only 5% of Lyme borreliosis patients.

Radiating pain (43 %), myalgia (41 %) and general fatigue (32 %) were the three most reported symptoms among patients diagnosed with Lyme borreliosis. The most reported symptoms among patients discharged without a specific diagnosis were general fatigue (60 %),

myalgia (53 %) and headache (39 %). Patients discharged without a specific diagnosis were significantly more likely to suffer from general fatigue (*p* = 0.001) and cognitive dysfunction (*p* = 0.04) and less likely to present with motor nerve palsy (*p* = 0.01) and radiating pain (*p* = 0.05). The comparison and clinical characteristics are displayed in Table 3.

4. Discussion

This prospective cohort study, including 215 adult patients referred under suspicion of tick-borne diseases, was conducted at the Clinic for Tick-borne Diseases at Rigshospitalet, Denmark 2017–2019. In total, one third were diagnosed with definite Lyme borreliosis, possible Lyme borreliosis or post-treatment Lyme disease syndrome. One third of the referred patients were diagnosed with a specific non-tick-associated disease, which underscore the importance of thoughtful investigation of patients referred under suspicion of a tick-borne disease.

4.1. Epidemiology and distribution

Slightly more women were examined and subsequently diagnosed with definite Lyme borreliosis than men, which is in concordance with other European studies (Petruioniene et al., 2020; Sajanti et al., 2017;

Eliassen et al., 2017; Bennet et al., 2007; Wilking and Stark, 2014; Rizzoli et al., 2011).

The majority of patients were referred by their general practitioner mostly due to (i) clinical suspicion of Lyme neuroborreliosis to be ruled out by lumbar punctures; (ii) uncertainty about interpretation of results of *B. burgdorferi* sl. serology; (iii) to ensure proper antibiotic therapy of previous manifestation of Lyme borreliosis or; (iv) uncertainty regarding manifestations of disseminated Lyme borreliosis combined with an increasing demand from the patients.

In accordance with the literature, Lyme borreliosis treatment and diagnostics give rise to public debate concerning difficulties managing both patient's wishes and evidence-based medicine (Jacquet et al., 2019).

We accepted referrals of patients with previous treated Lyme borreliosis. This, in order to ensure that patients are treated in accordance with national guidelines and are given evidence-based information regarding antibiotic therapy and disease prognosis. Centralizing patients with confirmed disseminated Lyme borreliosis may also be of importance to ensure a unified follow-up setting. A systematic follow-up is important both in order to deliver data to the scientific community and to patient for a regarding Lyme borreliosis. Also because the quality of information published on this topic in the press and at the internet is variable (Lantos et al., 2015; Sood, 2002).

4.2. Serodiagnostic tests cannot stand alone

A key point in the diagnosis of Lyme borreliosis is clinical symptoms and manifestations, relevant exposure and serology, when appropriate. Since antibodies can be present years after a well-treated infection and studies have proven that *B. burgdorferi* sl antibodies can be present in healthy individuals (Wilhelmsson et al., 2016; Lager et al., 2019; Kalish et al., 2001; Marangoni et al., 2008; Steere et al., 2008), serology cannot exclusively diagnose Lyme borreliosis if relevant symptoms are not present.

In total, one third was discharged without a specific diagnosis. Of those, one third had detectable *B. burgdorferi* sl IgG or IgM antibodies in serum. Of the patients, diagnosed with a non-tick-associated disease, 50 % had *B. burgdorferi* sl IgG or IgM antibodies present in serum. These results are supported by a Swedish study, which found that more than half of the seropositive subjects did not recall previous symptoms of Lyme borreliosis (Wilhelmsson et al., 2016), indicating that subclinical seroconversion is just as common as seroconversion accompanied by symptoms. Both results underline that serodiagnostic tests cannot stand alone in the diagnostic work-up and may cause considerable overdiagnosis.

We were able to establish a definite Lyme borreliosis diagnosis in one fifth of the referred patients, which is comparable to the numbers reported in Lyme borreliosis centers in other countries throughout Europe (Cottle et al., 2012; Jacquet et al., 2019; Coumou et al., 2015; Wilhelmsson et al., 2016).

A subgroup was identified as possible Lyme borreliosis, either possible Lyme neuroborreliosis or possible Lyme arthritis. The patients presenting with possible Lyme borreliosis were treated with antibiotics according to the European guidelines (Mygland et al., 2010; Stanek and Strle, 2018).

4.3. Antibiotic therapy has no impact on post-treatment Lyme disease syndrome

The cause of ongoing subjective symptoms after sufficient treatment for Lyme borreliosis has given rise to much speculation (Koedel et al., 2015). Twelve patients assessed in the Clinic for Tick-borne Diseases were diagnosed with post-treatment Lyme disease syndrome. All 12 patients had had previous definite Lyme neuroborreliosis and were previously treated according to the national guidelines (Dessau et al., 2014). To rule out ongoing disease, a repeated lumbar puncture was

performed. CSF investigations revealed no sign of ongoing disease.

Evidence suggests that a small subset of patients treated for Lyme borreliosis experience persisting symptoms despite antibiotic treatment (Kullberg et al., 2020; Wormser et al., 2006). By definition, patients with post-treatment Lyme disease syndrome have no clinical or laboratory support for ongoing *B. burgdorferi* sl infection, neither have they sign of irreversible nerve damage (Kullberg et al., 2020). Our data suggest the same, as we were not able to verify CSF inflammation in patients with persisting subjective symptoms 6 months after antibiotic cessation for Lyme neuroborreliosis.

Only little support for prolonged antibiotic treatment in patients with persistent symptoms due to Lyme borreliosis have been provided in the literature (Mygland et al., 2010; Kullberg et al., 2020). Hence, more research is needed to explore alternative treatment or efficiency of symptom-oriented therapy in these patients.

4.4. A high proportion of other diseases were found among patients referred under Lyme borreliosis suspicion

A non-tick-associated diagnosis was established in 33 % of the referred patients compared to 22 % in the Netherlands, 49 % in France and 33 % in the United Kingdom (Coumou et al., 2015; Jacquet et al., 2019; Cottle et al., 2012). The distribution of other medical conditions was alike in all countries and included both rheumatological and neurological diagnoses. In our cohort, we diagnosed patients with rheumatological diseases and three cases of cancer among other diagnoses. The high proportion of other medical conditions found in both our cohort and the above, underlines the need of careful medical examination and interdisciplinary collaboration.

4.5. Significantly more patients with Lyme borreliosis suffered from radiating pain and had a shorter duration of symptoms

Interestingly, patients without a specific diagnosis after ended assessment had significantly longer duration of symptoms prior to the first hospital visit than Lyme borreliosis patients. This finding is in accordance with a Norwegian study of 140 patients referred with suspected Lyme neuroborreliosis. In contrast to the 6 patients diagnosed with Lyme neuroborreliosis a large percentage of the patients with non-typical symptoms had had symptoms lasting more than 6 months (Roaldsnes et al., 2017).

Symptom presentation in Lyme borreliosis patients are diverse, they vary from a local rash (erythema migrans) to joint and heart involvement and even severe neurological symptoms. Despite the heterogeneity in symptoms, we found significant differences in the frequency of specific symptoms. Patients without a specific diagnosis after assessment, were significant more likely to suffer from general fatigue ($p=0.003$) than patients diagnosed with Lyme borreliosis. Patients with Lyme borreliosis were more likely to present with motor nerve palsy ($p = 0.01$) and radiating pain ($p = 0.05$) than patients without Lyme borreliosis. An assumption to this tendency would be the attribution from Lyme neuroborreliosis in the Lyme borreliosis group. Furthermore, the findings correlate well with the fact that objective neurological symptoms often have a somatic etiology, such as Lyme neuroborreliosis, but subjective symptoms such as general fatigue can be caused both by somatic, social and psychological problems and sometimes without an objective medical reason.

4.6. Thorough investigations in patients with a heavy symptom burden are of importance

Irrespective of a definite Lyme borreliosis diagnosis, patients referred to the Clinic for Tick-borne Diseases often suffered from a heavy symptom burden. Patients with Lyme borreliosis usually recover after relevant antibiotic treatment, but the patients without a confirmed diagnosis are, in other settings, often left without answers. In the matter

of handling patients with complex symptoms, multidisciplinary collaboration is of utmost importance. Therefore, clinics like the recently established “Center for Complex symptoms” at Frederiksberg Hospital in Copenhagen is appreciated. The multidisciplinary center is taking care of patients, who have functional limitations or who are suffering from anxiety disorders and the center is expected to play an important role in the further management of this complex and challenging group of patients.

Though, the patient’s symptoms are real, and in some cases disabling, no evidence support the use of antibiotics to non-infections. On the contrary, adverse effects have been reported in long term antibiotic use (Cadavid et al., 2016). The Clinic for Tick-borne Diseases could be a strong asset in unifying Lyme borreliosis treatment strategies and spread evidence-based information regarding test validation and evidence-based antibiotic treatment. Some patients might also need more thorough information on Lyme borreliosis, from an infectious disease specialist.

4.7. Strengths and limitations

The advantages of the present study were: (i) the same specialist in infectious diseases interviewed and examined all included patients; (ii) easy access for the general practitioners to refer patients; (iii) the recent inclusion of patients (2017–2019).

Limitations of the study were: (i) possible recall bias regarding tick exposure, previous erythema migrans etc.; (ii) a time-consuming study design (iii) small sample size in the Lyme borreliosis group; (iv) follow-up period is not long enough to determine if residual symptoms could be due to residual damage on the central nervous system.

5. Conclusion

In conclusion, we prospectively described the first 215 patients referred to the Clinic for Tick-borne Diseases at the department of Infectious Diseases at Rigshospitalet, Copenhagen. In total, 21 % of referred patients were diagnosed with Lyme borreliosis; 33 % were diagnosed with a non-tick-associated diagnosis after ended outpatient course and 1% with a tick-borne disease other than Lyme borreliosis. The most frequent diagnoses among the patients with a non-tick-associated diagnosis were rheumatological diseases and neurological diseases. Moreover, three were diagnosed with cancer. These findings underscore the importance of patients referred under suspicion of Lyme borreliosis benefit from being evaluated and investigated by a multidisciplinary approach in order to establish a diagnosis of Lyme borreliosis, an alternative diagnosis or to rule out ongoing disease.

Local ethics committee approval

The study is a quality assurance study and approved by local management. According to Danish law, approval from the Ethics committees is not required.

Declaration of Competing Interest

Outside of the present work: AML reports speakers honorarium/travel grants from Gilead, speakers honorarium/travel grants from GSK, travel grants from MSD and advisory board activity from Gilead. The other authors report no conflicts of interest.

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