

Borrelia burgdorferi sensu lato serology in the Netherlands: guidelines versus daily practice

J. Coumou · J. W. R. Hovius · A. P. van Dam

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Abstract The purpose of this study was to compare guideline recommendations and day-to-day practice of serological testing for Lyme borreliosis (LB) in a laboratory located in Amsterdam, the Netherlands, serving both regional hospitals and primary care physicians. By telephone interview, we obtained clinical information regarding 488 requests for LB serology. Screening for LB was performed with a C6-peptide EIA and confirmed by recombinant immunoblot. A total of 82 % of the requests were not supported by guideline's recommendations and either originated from patients with atypical symptoms and a low a priori chance for LB or from patients for which testing on LB was not recommended for other reasons. C6-EIA screening was positive in 5 % of patients with atypical symptoms, comparable to the seroprevalence in the Dutch population. Interestingly, 10 % of the requests were from patients with atypical skin lesions, of which 20 % was positive, suggesting that serological testing is of additional value in a selection of such patients. Strikingly, only 9 % of the requests were supported by recommendations by guidelines. The percentage of positive confirmatory IgM

and/or IgG immunoblots did not differ substantially between the groups and ranged from 56 to 75 %. Guidelines for testing for LB are not adequately followed in the Netherlands. Better education and adherence to the guidelines by physicians could prevent unnecessary diagnostics and antibiotic treatment of supposed LB patients.

Introduction

Lyme borreliosis (LB) is endemic in many European countries, as well as parts of the United States and Asia. In Europe, there are approximately 85,000 registered cases of LB each year [1]. LB is caused by *Borrelia burgdorferi* sensu lato spirochetes, which are primarily transmitted by *Ixodes ricinus* ticks [2]. *Borrelia garinii* and *Borrelia afzelii* and—to a lesser extent—*Borrelia burgdorferi* are the predominant causative agents [3].

In 2011, the European society of clinical microbiology and infectious diseases (ESCMID) study group for Lyme borreliosis (ESGBOR) recommended LB case definitions for diagnostic testing and treatment which are available on the website www.eucalb.com [4]. These recommendations are in line with other national guidelines, such as the Dutch health organization “Centraal Begeleidings Orgaan” (CBO) guideline from 2013 and other European and international guidelines, including the guideline published in 2006 by the Infectious Disease Society of America (IDSA) [5–8]. According to these guidelines, the diagnosis of LB is based on the presence of specific symptoms, and—when appropriate (see below)—combined with positive serological and/or other diagnostic tests. On average 1–2 weeks after a tick bite, an infection with *B. burgdorferi* s.l. can lead to an expanding erythematous skin lesion, in most patients with central clearance, designated as erythema migrans (EM). In case of a typical EM, no further testing is recommended since clinical symptoms can precede

J. Coumou · A. P. van Dam
Public Health Laboratory, Amsterdam Health Service, 1090
HM Amsterdam, The Netherlands

J. Coumou (✉) · J. W. R. Hovius
Center for Experimental and Molecular Medicine, Academic
Medical Center Amsterdam, University of Amsterdam,
Meibergdreef 9, Amsterdam 1105 AZ, The Netherlands
e-mail: j.coumou@amc.nl

J. W. R. Hovius
Academic Medical Lyme Center, Academic Medical Center
Amsterdam, University of Amsterdam, Amsterdam 1105 AZ, The
Netherlands

A. P. van Dam
Department of Medical Microbiology, Onze Lieve Vrouwe Gasthuis,
1091 AC Amsterdam, The Netherlands

the antibody response [9, 10]. If left untreated, spirochetes can disseminate (weeks to months) and cause inflammation of other organs, leading to lymphocytoma, multiple erythema migrans, neuroborreliosis, Lyme arthritis or acrodermatitis chronica atrophicans, amongst other rare manifestations. To establish the diagnosis of disseminated LB, laboratory evidence of a *Borrelia* infection is required. A detailed overview of the case definitions for LB is available at www.eucalb.com and in other guidelines and position papers [4, 8]. In the absence of symptoms compatible with disseminated LB, in general the guidelines recommend not to test for LB, because of the low positive predictive value (PPV) of the serological tests in this setting. In addition, the guidelines state that serological testing is not recommended to confirm the efficacy of antibiotic treatment of a (suspected) *Borrelia* infection, since antibodies might remain detectable for many years, even in the absence of symptoms [5, 8].

For serologic testing on LB, European guidelines recommend that (at least second generation) enzyme-linked immunosorbent assays (ELISAs or EIAs) targeting all European *Borrelia* species should be used as a screening test and, when reactive, should be confirmed by an IgM/IgG immunoblot with a specificity of at least 95 % (two-tier testing) [4, 5]. A broad spectrum of *Borrelia burgdorferi* s.l. antigens, e.g. OspC, VlsE, p100 and p18, should be present in the immunoblot. The confirmation by immunoblot is required to distinguish between true positive EIA results and aspecific positive results in EIAs. A recent improvement of serology was obtained by the inclusion of the C6 peptide in EIAs, which represents a constant and conserved region of the VlsE protein [11], and could possibly be used as the only antigen to which antibodies against *Borrelia* can be measured [12].

In practice, the interpretation of serologic results by physicians is complicated by the occurrence of both false-positive and false-negative findings. False-negative results are frequently found in early LB, especially EM. The frequency of false-negative findings in late LB has reported to be extremely low [9]. False-positive findings can be caused by preceding symptomatic infection and specificity problems of assays, caused by cross-reactivity due to rheumatoid factors, acute EBV, CMV or *Treponema pallidum* infections, multiple sclerosis and other auto-immune diseases [13]. Furthermore, (endured) asymptomatic *Borrelia* infections can also lead to positive antibody responses. Therefore it is not surprising that around 4–20 % of the Western-European population has (specific) antibodies against *Borrelia* [14–17]. In clinical practice, false-positivity, rather than actual LB, is much more likely to account for positive test results in individuals without suggestive symptoms for LB [18]. Therefore, the Dutch 2013 CBO guideline, among other guidelines, highlights the low PPV of the presence of antibodies against *Borrelia* in the absence of symptoms suggestive of LB [5].

Even though the recommendations for serological testing for LB are clear, they do not always translate into practice. One reason for this might be that many atypical symptoms have been ascribed to LB, such as fatigue, headache, or cognitive deficits including loss of memory. This could be a reason for serological testing by physicians, sometimes explicitly requested by the patient, also in the absence of specific symptoms related to LB. Alternatively, physicians might not be aware of the existing guidelines. The aim of the present study was to obtain data about the population screened for LB in a laboratory serving both regional hospitals and primary care physicians and to illustrate the difference between guideline recommendations and actual clinical practice on testing for LB in the Netherlands.

Materials and methods

Serological tests

The study was performed in two laboratories in which sera from four hospitals and a large number of general practitioners (GPs) are submitted to test for antibodies against *Borrelia*. In both laboratories, the C6-EIA (Immunitics, Boston, USA) was used as a screening test. For the purpose of this study samples were considered positive when the Lyme-index (sample OD/cut-off OD, according to the manufacturer's instructions) was above 1. Positive samples were subsequently tested in an IgG and IgM recombinant immunoblot (Mikrogen, Neuried, Germany) as confirmatory tests, according to the manufacturer's instructions and were classified as negative, indeterminate or positive.

Patient population

To obtain clinical data regarding patients from which serum samples were submitted, a single researcher (JC) performed telephone interviews with submitting physicians. Information on the type of symptoms, length of symptoms, previous serological testing, antibiotic treatment and a history of tick bites was collected through a standardized questionnaire. Furthermore, an additional effort was done to obtain clinical data on patients who had a positive result in the screening EIA. For a more detailed overview of the selection process see the results sections (Fig. 1). The study was performed from February 15th to September 1st, 2010. For further analysis ESGBOR criteria were used to classify individuals (Table 1).

Database analysis

All analysis was performed with SPSS software version 20.

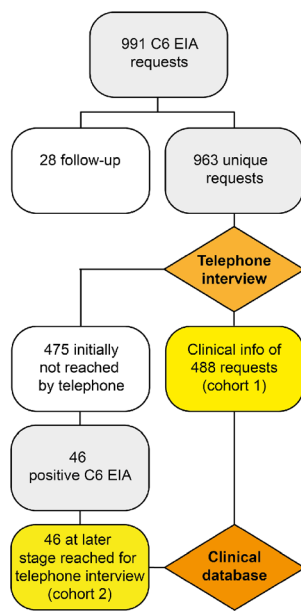


Fig. 1 Flowchart of data collection. The study was performed from February 15th to September 1st, 2010. To obtain clinical data regarding patients from which serum samples were submitted, a single researcher (JC) performed telephone interviews with submitting physicians

Results

During the study period, 963 sera from newly tested unique patients were sent to the participating laboratories for *Borrelia* testing (Fig. 1). From 488 patients, clinical data were obtained by telephone interview irrespective of the test result (cohort 1). In addition, of the remaining 475 patients, the clinical data could also be obtained from 46 patients (cohort 2) with a positive C6 EIA test result.

Table 1 Definition of clinical groups

Current EM	Erythema migrans diagnosed by a physician
Symptoms compatible with disseminated LB	Symptoms which are characteristic for disseminated LB according to ESGBOR (e.g. arthritis, facial nerve paresis, ACA)
Atypical symptoms after possible EM	History of EM (not confirmed by a physician) for which no treatment was given. Present symptoms are atypical for LB
Atypical symptoms after treated EM	History of EM for which recommended treatment was given. Present symptoms are atypical for LB
Atypical skin lesion	Skin lesions atypical for EM as determined by the submitting physician
Atypical symptoms, no EM	No history of EM, symptoms are atypical for LB
Asymptomatic, recent tick bite	No symptoms, but recent tick bite

ACA Acrodermatitis chronica atrophicans, EM erythema migrans, ESGBOR European society of clinical microbiology and infectious diseases (ESCMID) study group for Lyme borreliosis, LB Lyme borreliosis

Of the 488 patient of cohort 1 most samples were sent from GP offices (72 %) (Table 2). Individuals were divided into distinct clinical categories based on the obtained clinical information and according to the ESGBOR criteria (Table 1). Of the 488 patients 23 patients (5 %) presented with an EM according to the clinician, 32 (7 %) had symptoms compatible with disseminated LB according to ESGBOR criteria, and 49 (10 %) had skin lesions that could be ascribed to LB, but were not typical for EM according to the clinician. Another subgroup consisted of nine patients (2 %) who reported a previous EM, not confirmed by a physician, for which no treatment was given and were now presenting with new symptoms, however not typical for LB. In contrast, 34 patients (7 %) in our database were diagnosed in the past with an EM and had received antibiotic treatment and now presented with new symptoms, not typical for LB. By far the largest group in cohort 1, 322 patients (66 %), had symptoms not typical for LB, such as fatigue, headache, myalgia and arthralgia. Finally, there were 19 requests (4 %) from individuals without any symptoms, but who had experienced a recent tick bite. Even in the presence of a positive *Borrelia* serology, the diagnosis LB would still be questionable in these last two subgroups, comprising 341 patients and thus 70 % of cohort 1.

All sera were initially tested in a C6-EIA as a screening test. A positive screening test was found in 17 of the 23 patients (74 %) with an EM (Table 2). In only five of the 32 patients (16 %) with symptoms fulfilling clinical criteria for disseminated LB according to ESGBOR criteria we found a positive C6-EIA. In the screening test, we found that 22 % was positive in the group with non-confirmed untreated EM in the past and that 29 % of patients with a confirmed treated EM in their medical history were positive. Notably, 20 % of the patients with atypical skin lesions also had a positive C6-EIA. In the group with atypical clinical symptoms, only 5 % had a positive C6 screening test, which is comparable to the seroprevalence in the Dutch population [17]. All of the 19 individuals without symptoms after a recent tick bite and with concerns of LB had a negative screening test.

Clinical data was also obtained from 46 patients whose physicians had been contacted after the positive result of the screening test was known (cohort 2, Fig. 1). All sera from cohorts 1 and 2 that had a positive C6 EIA were tested in an IgM and IgG recombinant immunoblot. The percentage of positive IgM and/or IgG blots did not differ substantially between the groups and ranged from 56 to 75 % (Table 3), excluding the group with atypical symptoms after a possible EM, since there were only three samples with a positive C6 EIA in this group (100 % positive IgG and/or IgM). Most indeterminate blots were seen in the group with EM and treated EM. Notably, the percentage of positive IgM immunoblots was higher in the group with EM and symptoms compatible with LB (both 63 %) compared to the group with atypical symptoms (38 %). In contrast, when patients with

Table 2 Patient characteristics of cohort 1

Clinical group	Requests <i>n</i> (%)	Sex Female, <i>n</i> (%)	Age Mean (range)	Duration of symptoms > 6 months, <i>n</i> (%)	Specialism of requesting physician				C6 EIA		
					GP	Internal medicine	Neurology	Dermatology	Pediatrics	Other specialism	Positive
Current EM	23 (5 %)	14 (61 %)	45 (55)	0 (0 %)	19 (83 %)	1 (4 %)	0 (0 %)	3 (13 %)	0 (0 %)	0 (0 %)	17 (74 %)
Symptoms compatible with disseminated LB	32 (7 %)	17 (53 %)	53 (67)	4 (13 %)	4 (13 %)	1 (3 %)	11 (34 %)	3 (9 %)	5 (16 %)	8 (25 %)	5 (16 %)
Atypical symptoms after possible EM	9 (2 %)	9 (100 %)	51 (40)	5 (56 %)	6 (67 %)	0 (0 %)	1 (11 %)	1 (11 %)	0 (0 %)	1 (11 %)	2 (22 %)
Atypical symptoms after treated EM	34 (7 %)	18 (53 %)	50 (61)	12 (36 %)	28 (82 %)	2 (6 %)	4 (12 %)	0 (0 %)	0 (0 %)	0 (0 %)	10 (29 %)
Atypical skin lesion	49 (10 %)	27 (55 %)	44 (76)	3 (7 %)	36 (74 %)	2 (4 %)	0 (0 %)	10 (20 %)	1 (2 %)	0 (0 %)	10 (20 %)
Nonspecific or no symptoms, no EM	322 (66 %)	174 (54 %)	42 (89)	155 (48 %)	241 (75 %)	19 (6 %)	34 (11 %)	1 (0 %)	1 (2 %)	16 (5 %)	17 (5 %)
Asymptomatic, recent tick bite	19 (4 %)	12 (65 %)	43 (66)	NA	19 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Total	488 (100 %)	271 (56 %)	43 (90)	179 (37 %)	353 (72 %)	25 (5 %)	50 (10 %)	18 (4 %)	17 (3 %)	25 (5 %)	61 (13 %)

Only patients of cohort 1, in which clinical data were obtained by telephone interview irrespective of the screening test result, are shown. Patients were divided into groups according to criteria shown in Table 1. EIA enzyme immuno assay, EM erythema migrans, GP general practitioner, LB Lyme borreliosis, NA not applicable

atypical symptoms had a positive immunoblot, this was frequently IgG, which could be compatible with a *B. burgdorferi* s.l. infection in the past.

Of the total 963 unique requests sent to our laboratories, 107 had a positive C6 screening test. Of those 107, 56 patients (52 %) had symptoms compatible for LB (Fig. 2), according to ESGBOR criteria. Of those 56, 24 (22 % of positive screening tests) had a current EM and 16 (15 %) had symptoms compatible with disseminated LB. In three of the 16 patients with symptoms that were compatible with disseminated LB, the diagnosis LB was finally discarded after a negative confirmatory immunoblot. These 56 patients also included 16 patients (15 %) with an atypical skin lesion and a confirmatory immunoblot when symptoms lasted longer than 2 months. The other 51 positive screening tests (48 %) were from patients with atypical symptoms for LB.

Discussion

Our study clearly shows that the far majority of sera sent to our laboratories—72 % coming from GPs—originate from patient populations in which *B. burgdorferi* s.l. serology has a low PPV for LB. Based on our study, we estimate that 82 % of the requests sent to our laboratories are not supported by recommendations in established guidelines, i.e. requests for patients with typical EM (5 %), for patients with atypical symptoms (73 %), or for patients having no symptoms at all (4 %). The requests that were based on guideline recommendations consisted of symptoms compatible with disseminated LB (7 %), an untreated EM in the past (2 %) and requests for laboratory support for an atypical skin lesion (10 %). Apparently, due to anxiety concerning ‘chronic LB’ in the Netherlands, many patients are tested in the absence of clinical symptoms compatible with LB. Despite a low a priori chance of LB, a positive serological test might make a physician consider antibiotic treatment, which is unlikely to result in cure of the patient when LB is not the cause of the symptoms, and has the risk of complications and delayed treatment of the actual diagnosis. Whether false positive tests in our database actually lead to unnecessary antibiotic treatment or incorrect diagnosis is unknown and—to our knowledge—there are no other data available on this issue. Since many guidelines, i.e. the European ESGBOR, the North-American IDSA and the Dutch CBO are [4, 5, 8], to a large extent, in accordance with each other on two-tier testing and case definitions, and since the incidence of LB in the Netherlands is similar to other European countries, our results most likely could be extrapolated to other European countries [3, 6, 7, 19].

According to LB criteria as described by the guidelines, the PPV of the C6 screening test in our population (cohort 1 and 2) is 50 % (53/107) (Fig. 2). In this database, 51 (5 %) of the total unique requests came from patients with atypical symptoms

Table 3 Immunoblot confirmation test results of C6 positive samples

Clinical group	C6-EIA positive samples		IgM Immunoblot		IgG Immunoblot		Positive IgM and/or IgG immunoblot	Indeterminate IgM and/or IgG immunoblot						
			Positive	Indeterminate	Positive	Indeterminate								
Current EM	24	(22 %)	15	(63 %)	2	(8 %)	7	(29 %)	10	(42 %)	18	(75 %)	4	(17 %)
Symptoms compatible with disseminated LB	16	(15 %)	10	(63 %)	0	(0 %)	5	(31 %)	5	(31 %)	11	(69 %)	1	(6 %)
Atypical symptoms after possible EM	3	(3 %)	2	(67 %)	0	(0 %)	2	(67 %)	1	(33 %)	3	(100 %)	0	(0 %)
Atypical symptoms after treated EM	16	(15 %)	0	(0 %)	0	(0 %)	10	(63 %)	4	(25 %)	10	(63 %)	4	(17 %)
Atypical skin lesion	16	(15 %)	6	(38 %)	0	(0 %)	4	(25 %)	5	(31 %)	9	(56 %)	2	(13 %)
Atypical or no symptoms, no EM	32	(30 %)	6	(19 %)	2	(6 %)	18	(56 %)	4	(13 %)	22	(69 %)	1	(3 %)
Asymptomatic, recent tick bite	0	(0 %)												
Total	107	(100 %)	39	(36 %)	4	(4 %)	46	(43 %)	29	(27 %)	73	(68 %)	12	(10 %)

Patients of both cohorts were divided into groups according to criteria (Table 1). Immunoblots were performed in patients with a positive C6 EIA screenings test from cohort 1 and 2. EM erythema migrans, LB Lyme borreliosis

and a positive screening test. However, it cannot completely be excluded that atypical symptoms in a seropositive patient in the absence of characteristic clinical signs are caused by active LB, and therefore an absolute PPV cannot be provided.

In the group of patients with an EM, 74 % had positive serology. Although this is higher than reported in other studies [20, 21], this is in line with the delayed antibody responses in such patients. Interestingly, we found anti-*B. burgdorferi* s.l. antibodies in 90 % of patients with a current EM lasting for four weeks or longer (n=17) compared to 60 % in patients with symptoms shorter than 4 weeks (n=12). Another interesting finding is the relatively high frequency of positive test results in the group of patients with atypical skin lesions. The confirmatory immunoblot was positive in 9/16 patients (56 %).

However, of the remaining seven patients, six had symptoms lasting shorter than 2 months, in which an immunoblot

can still be false-negative, and one patient had an indeterminate IgG immunoblot despite symptoms lasting longer than 2 months. Serologic results combined with the obtained clinical data indicate that of these 16 patients, 14 probably had an EM and two probably had a lymphocytoma. Since we do not have photographic documentation of these lesions, it is not clear whether these lesions were more or less typical, but not clinically recognized by physicians, or whether they were atypical and therefore not recognized as such. Since the initial sensitivity of serology in our patients with typical EM was ‘only’ 74 %, a number of patients with atypical skin lesions due to early LB could remain undiagnosed and untreated due to seronegativity. In this study, this would be the case for six patients. However, since the duration of symptoms among patients with atypical lesions (median of 2 months) was longer compared to the EM group (median of 1 month), false-negative antibody responses are less likely in this group. Nonetheless, serological testing might be of additional value in a selection of such patients. Interestingly, a study from Denmark also showed an increased frequency of antibodies against *Borrelia* in patients with skin rashes [22]. A notable difference between the Danish study and ours was that in Denmark 38 % of patients presented with skin rashes, and only 26 % had nonspecific or no symptoms. This may suggest that in Denmark testing is more often restricted to patients with clinical symptoms compatible with LB.

A history of tick bites can be part of the rationale for the decision to perform serological testing for LB. However, we found that in the group with atypical symptoms and no report of EM, 51 % did not witness any tick bites, compared to 31 % that did report tick bites. Interestingly, in 29 individuals (9 %) who had reported tick bites preceding the onset of the atypical symptoms, 5 (17 %) had a positive screening test and a positive IgG and/or IgM immunoblot. However, three out of five of these samples came from forestry workers, who are highly exposed to tick bites (data not shown).

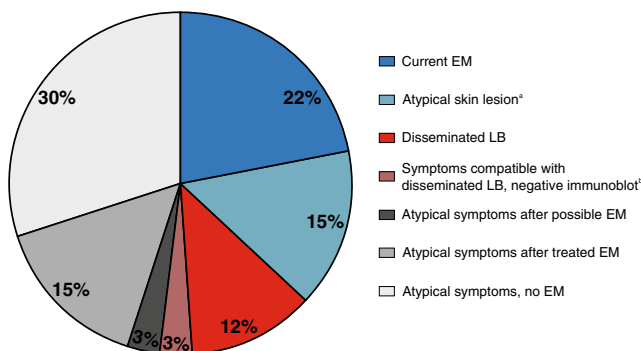


Fig. 2 Distributions of positive C6 EIA results (n=107) among different clinical groups of cohort 1 and 2. Criteria of the clinical groups are found in Table 1. The group “Asymptomatic, recent tick bite” did not have any positive tests and is therefore not shown. ^a Including one patient with an indeterminate immunoblot and symptoms longer than 2 months ^b Symptoms compatible with LB with a positive C6 EIA result; however, the diagnosis LB was finally discarded after a negative confirmatory immunoblot

A recent expert meeting of the European Centre for Disease Control on laboratory diagnosis of LB recommended an improved dialogue between clinicians and medical microbiologist about the difficulties that both groups face when dealing with LB. With this study we hope to contribute to this dialogue by describing the patient population in which Dutch physicians consider LB in their differential diagnosis. Only in few cases, serological testing contributed to the final diagnosis of LB. Apparently, many physicians perform serological testing for LB on individuals with a low a priori chance of LB. Although a negative result lowers the suspicion of LB, the fact that around 5–10 % of these cases will be positive due to either false-positivity or previous exposure to *B. burgdorferi* s.l unrelated to the current clinical symptoms could lead to over diagnosis of LB. Future tests that can better distinguish between past and current infection would contribute to improved care for patients suspected of LB. Until such tests are available, we recommend better implementation of current guidelines and more education on the low PPV of current serologic tests for LB when not cautiously used. This will lower costs and prevent unnecessary antibiotic treatment.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Lindgren E, Jaenson TGT (2006) Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. <http://www.euro.who.int/en/health-topics/environment-and-health/Climate-change/publications/pre-2009/lyme-borreliosis-in-europe.-influences-of-climate-and-climate-change,-epidemiology,-ecology-and-adaptation-measures>. Accessed 06 May 2014
- Steere AC (1989) Lyme disease. *N Engl J Med* 321(9):586–596
- Strle F, Stanek G (2009) Clinical manifestations and diagnosis of Lyme borreliosis. *Curr Probl Dermatol* 37:51–110. doi: 10.1159/000213070
- Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, Kristoferitsch W, O'Connell S, Ornstein K, Strle F, Gray J (2011) Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect* 17(1):69–79. doi: 10.1111/j.1469-0691.2010.03175.x
- CBO Guideline Lymeziekte. <http://www.diliguide.nl/document/1314>
- Vanousova D, Hercogova J (2008) Lyme borreliosis treatment. *Dermatol Ther* 21(2):101–109. doi: 10.1111/j.1529-8019.2008.00177.x
- Flisiak R, Panczewicz S (2008) Diagnostics and treatment of Lyme borreliosis. *Recomm Pol Soc Epidemiol Infect Dis Przegł Epidemiol* 62(1):193–199
- Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, Krause PJ, Bakken JS, Strle F, Stanek G, Bockenstedt L, Fish D, Dumler JS, Nadelman RB (2006) The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 43(9): 1089–1134. doi: 10.1086/508667
- Steere AC, McHugh G, Damle N, Sikand VK (2008) Prospective study of serologic tests for Lyme disease. *Clin Infect Dis* 47(2):188–195. doi: 10.1086/589242
- Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJ, Wormser GP, Steere AC (2010) 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test. *Clin Infect Dis* 50(1): 20–26. doi: 10.1086/648674
- Liang FT, Steere AC, Marques AR, Johnson BJ, Miller JN, Philipp MT (1999) Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* vlsE. *J Clin Microbiol* 37(12):3990–3996
- Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC (2013) Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. *Clin Infect Dis* 57(3):333–340. doi: 10.1093/cid/cit235
- Wilske B, Fingerle V, Schulte-Spechtel U (2007) Microbiological and serological diagnosis of Lyme borreliosis. *FEMS Immunol Med Microbiol* 49(1):13–21. doi: 10.1111/j.1574-695X.2006.00139.x
- Gutierrez J, Guerrero M, Nunez F, Soto MJ, Piedrola G, Maroto MC (2000) Antibodies to *Borrelia burgdorferi* in European populations. *J Clin Lab Anal* 14(1):20–26. doi: 10.1002/(SICI)1098-2825(2000)14:1
- Fahrer H, van der Linden SM, Sauvain MJ, Gern L, Zhioua E, Aeschlimann A (1991) The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. *J Infect Dis* 163(2):305–310
- Carlsson SA, Granlund H, Nyman D, Wahlberg P (1998) IgG seroprevalence of Lyme borreliosis in the population of the Åland Islands in Finland. *Scand J Infect Dis* 30(5):501–503
- Nohlmans MK, van den Bogaard AE, Blaauw AA, van Boven CP (1991) Prevalence of Lyme borreliosis in The Netherlands. *Ned Tijdschr Geneesk* 135(48):2288–2292
- Lakos A, Reiczigel J, Solymosi N (2010) The positive predictive value of *Borrelia burgdorferi* serology in the light of symptoms of patients sent to an outpatient service for tick-borne diseases. *Inflamm Res* 59(11):959–964. doi: 10.1007/s00011-010-0209-1
- Wilske B (2005) Epidemiology and diagnosis of Lyme borreliosis. *Ann Med* 37(8):568–579. doi: 10.1080/07853890500431934
- Strle F, Nadelman RB, Cimpenman J, Nowakowski J, Picken RN, Schwartz I, Maraspin V, Aguero-Rosenfeld ME, Varde S, Lotric-Furlan S, Wormser GP (1999) Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. *Ann Intern Med* 130(1):32–36. doi: 10.7326/0003-4819-130-1-199901050-00006
- Stanek G, Breier F, Menzinger G, Schaar B, Hafner M, Partsch H (1999) Erythema migrans and serodiagnosis by enzyme immunoassay and immunoblot with three borrelia species. *Wien Klin Wochenschr* 111(22–23):951–956
- Dessau RB, Bangsberg JM, Ejlertsen T, Skarphedinsson S, Schonheyder HC (2010) Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: survey of patients seen in general practice. *BMC Infect Dis* 10:317. doi: 10.1186/1471-2334-10-317