Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Research Article

Seroprevalence of *Borrelia*-specific IgG and IgM Antibodies in Patients with Generalized Musculoskeletal Pain and/or Suspected of Lyme Borreliosis: A Two-tier Screening Approach Over a Two-year Period

Castelain S[1,2]*, Choquet M[1,2], Rossi F[3], Pluquet E[1,2], Schmit JL[2,3], Guiheneuf R[1,2] and Lanoix JP[2,3]

[1]Department of Bacteriology, Amiens University Medical Center, Amiens, France

[2]Microbiology Research Unit, AGIR, UR4294, Jules Verne University of Picardie, Amiens, France

[3]Department of Infectious Diseases, Amiens University Medical Center, Amiens, France

***Corresponding author:** Sandrine Castelain, Department of Bacteriology, Amiens University Medical Center, CBH, CHU Amiens-Picardie, F-80054 Amiens cedex, France

Received: August 11, 2020; **Accepted:** September 29, 2020; **Published:** October 06, 2020

Abstract

Lyme disease is challenging to diagnose because of nonspecific and heterogeneous clinical symptoms, and imperfect serologic assays. The objective of the present two-year study was to determine the seroprevalence of anti-*Borrelia* antibodies in a population of patients consulting for pain compatible with a suspected Lyme disease. Two-tier screening was performed on 2088 individuals. The seroprevalence of the anti-*Borrelia* antibodies was 6.0%. The presence of anti-*Borrelia* IgMs alone was predominantly observed in younger women and IgGs alone was predominantly observed in older men. Anti-*Borrelia* IgGs alone and IgMs alone were detected in respectively 15.1% and 3.2% of the patients with an initial clinical diagnosis of Lyme disease. In light of the observed seroprevalence, the high proportion of false positives, and the low proportion of true positives, we consider that a serologic assay should not be prescribed as a first-line diagnostic test. Interpretation of results should be done within a multidisciplinary team.

Keywords: Lyme disease; *Borrelia* burgdorferi sensu lato; Serology; Western blot; ELISA; Screening

Background

Lyme disease (also known as Lyme borreliosis) is the most frequent tick-borne disease in France and other European countries [1]. The Sentinel disease monitoring network estimated that the mean annual incidence of Lyme borreliosis in metropolitan France between 2009 and 2017 was 53 per 100,000 inhabitants [2]. Neighboring countries with similar surveillance networks have reported similar values. Lyme borreliosis is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex [3]. The causative agents of Lyme borreliosis in Europe are *Borrelia burgdorfer*i sensu stricto, *Borrelia garinii* and *Borrelia afzelii* [2]. *Borrelia* burgdorferi sensu stricto is predominant in the United States [4].

In this context, the presence of anti-*Borrelia* antibodies is a diagnostic hallmark of Lyme borreliosis. However, the performance of diagnostic tests for Lyme disease depends on the clinical presentation [5,6]. Indeed, the time course of the antibody response is relatively slow. Diagnosis at a localized stage of the disease (e.g. erythema migrans) should not therefore be based on serologic tests. In disseminated stages, seroconversion (the appearance of IgGs) occurs after about six weeks [7]. Furthermore, anti-*Borrelia* antibodies can persist for years after the clinical signs and symptoms have resolved [8]. Conversely, cross-reactivity and low test specificity can produce false-positive results [9]. Lastly, geographical differences in the epidemiology of *Borrelia burgdorferi* sensu lato within Europe may influence assay performance.

Many different antibody-based assays for *Borrelia burgdorferi* sensu lato are commercially available, and the choice of assay strongly influences the corresponding conclusions. To avoid pitfalls, all the current national, Europe-wide and North American evidence-based guidelines recommend a two-tier serologic approach to the diagnosis of Lyme borreliosis [10]. Specifically, immunoblots are used as a second-tier assay approach after positive or equivocal results have been detected in a first-tier Enzyme Immunoassay (EIA).

The clinical manifestations of Lyme disease are heterogeneous and can affect several organs, including the skin, the central nervous system, and the joints [7]. Over the past years, several researchers in the USA and Europe have suggested that many other symptoms could be due to a "chronic" infection caused by *Borrelia burgdorferi* sensu lato and that is not detected by currently available diagnostic methods [10,11]. Therefore, patients with chronic pain are increasingly consulting infectious disease specialists and asking to be screened with diagnostic tests for Lyme disease. Reliable serologic screening is therefore critically important for managing these patients.

The objective of the present two-year study was to determine the seroprevalence of specific anti-*Borrelia* antibodies in a population of patients consulting for symptoms compatible with disseminated Lyme borreliosis.

Materials and Methods

Patients and Specimens

We performed a single-center, retrospective study of patients admitted to Amiens University Medical Center (Amiens, France)

Citation: Castelain S, Choquet M, Rossi F, Pluquet E, Schmit JL, Guiheneuf R, et al. Seroprevalence of *Borrelia*-specific IgG and IgM Antibodies in Patients with Generalized Musculoskeletal Pain and/or Suspected of Lyme Borreliosis: A Two-tier Screening Approach Over a Two-year Period. J Bacteriol Mycol. 2020; 7(7): 1155.

Table 1: Demographic characteristics of 2088 patients having been screened with serologic assays for Lyme disease.

	Overall screening	Negative results	Positive or equivocal results for IgG and/or IgM
Number (%)	2088 (100)	1857 (88.9)	231 (11.1)
Sex			
Male (%)	956 (45.8)	859 (46.3)	97 (42.0)
Female (%)	1132 (54.2)	998 (53.7)	134 (58.0)
Age (years, mean ± SD)	48.4 ± 20.6	48.4 ± 20.7	47.9 ± 20.1
Patient's location at the time of testing (%)			
Inpatient unit	1419 (68.0)	1285 (69.2)	134 (58.0)
Outpatient unit	543 (26.0)	463 (25.0)	80 (34.6)
Emergency department	126 (6.0)	109 (5.8)	17 (7.4)

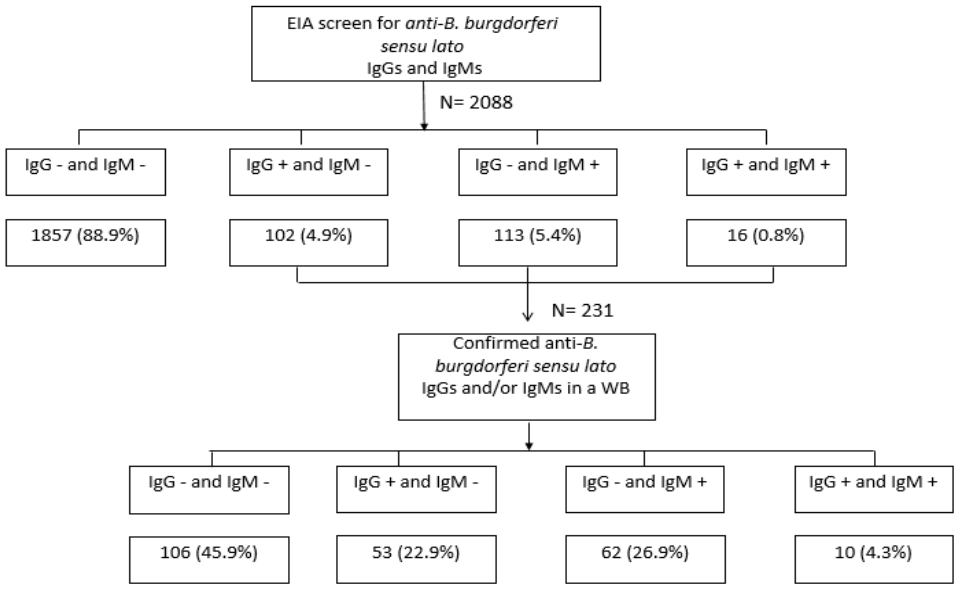

Figure 1: The presence of serum anti-*Borrelia* IgG and IgM antibodies in patients screened with a two-tiered approach between August 2016 and July 2018.

with signs, symptoms or clinical circumstances that were compatible with or suggestive of Lyme disease. Samples from 2088 individuals were collected between August 2016 and July 2018. All samples were stored at -70°C and thawed immediately before testing.

The First-Tier Screening Assays

First-tier screening for *Borrelia burgdorferi* sensu lato was performed with the LIAISON® *Borrelia* IgM II and *Borrelia* IgG (Diasorin, France) automated chemiluminescent EIAs. The LIAISON® *Borrelia* IgM II assay contains two recombinant antigens: the outer surface protein OspC (p25), which is immunodominant for the IgM response in the early phase of infection, and the variable major protein-like sequence VlsE, which has a major role in the immune response to Lyme borreliosis. The LIAISON® *Borrelia* IgG assay contains the recombinant *Borrelia* VlsE antigen.

The Second-Tier (confirmatory) Screening Assays

Confirmatory testing was performed with Euroline-WB *Borrelia* IgG and Euroline-WB *Borrelia* IgM Western blot assays (EUROIMMUN, Germany). Both assays contain whole-antigen, SDS extracts of *Borrelia afzelii*, and recombinant VlsE.

Statistical Analysis

Intergroup comparisons were performed with a non-parametric Mann-Whitney test for continuous variables, and a chi-squared test or Fisher's exact test for categorical variables. The threshold for statistical significance (two-tailed) was set to $p \leq 0.05$.

Ethics Statement

This was a non-interventional study; all diagnostic, monitoring and treatment procedures were part of routine patient care. Data were analyzed after that had been anonymized. In line with the French legislation on non-interventional clinical research, the study did not require approval by an institutional review board or the provision of informed consent by the participants.

Results

Two-Tier Screening

The results of the two-tier screening are summarized in Figure 1 and Table 1. Of the 2088 tests performed, 1857 were negative (88.9%) after first-tier EIA screening. There were three serologic profiles for the positive results: (i) positivity for IgG alone, accounting for 102 of the 231 positive EIAs (44.1%), (ii) positivity for IgM alone,

Table 2: The IgM and IgG index in the first-tier EIA, as a function of the results in the second-tier Western blot assay.

IgM (EIA index)		Non-confirmed result	Confirmed result	p
0.9 – 1.1	Number (%)	25 (49.0)	26 (51.0)	0.41
N = 51	Mean [95% CI]	0.94 [0.85 - 1.03]	1.03 [1.01- 1.05)	
1.2 – 2.0	Number (%)	19 (35.2)	35 (64.8)	0.11
N = 54	Mean [95% CI]	1.30 [1.23 - 1.38)	1.48 [1.40 - 1.56)	
>2.0	Number (%)	13 (54.2)	11 (45.8)	0.17
N = 24	Mean [95% CI]	3.66 [2.80 - 4.52)	2.92 [2.29 - 3.55)	
Total (%)		57 (44.2)	72 (55.8)	0.7
IgG (EIA UA/mL)		No confirmed assay	Confirmed assay	p
10 – 15	Number (%)	28 (75.6)	9 (24.4)	0.091
(N=37)	Mean [95% CI]	12.03 [11.46 - 12.60]	12.68 [11.87 - 13.49]	
>15 – 100	Number (%)	24 (38.7)	38 (61.3)	0.002
(N=62)	Mean [95% CI]	27.35 [22.28 - 32.43]	40.64 [33.87 - 47.40]	
>100	Number (%)	3 (15.8)	16 (84.2)	0.943
(N=19)	Mean [95% CI]	196.40 [109.2 - 240.0]	218.40 [203.1 - 233.8]	
Total (%)		55 (46.6)	63 (53.4)	<0.0001

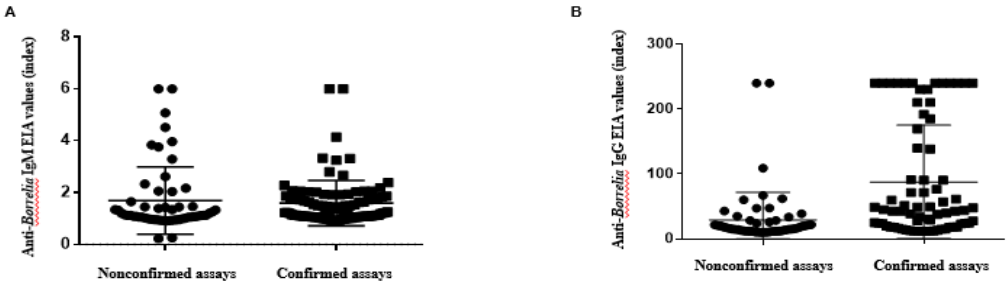

Figure 2: The EIA test values for anti-*Borrelia* IgM antibodies (A) and anti-*Borrelia* IgG antibodies (B), as a function of the result of the confirmatory Western blot assay.

accounting for 113 positive EIAs (48.9%), and (iii) positivity for both IgG and IgM, accounting for only 16 positive EIAs (6.9%). In line with the European and North American evidence-based guidelines, immunoblots were used as the second-tier assay after first-tier screening with EIAs had produced positive or equivocal results. In the second-tier screen, 53 of the 125 positive samples (42.4%) contained IgG alone and 62 (49.6%) contained IgM alone. Lastly, only 10 of the 16 first-tier IgG+ IgM+ samples were confirmed (62.5%). Ultimately, the seroprevalence of anti-*Borrelia* antibodies in the study population was 6.0% (125 out of 2088).

A detailed analysis of the EIA results showed that the anti-*Borrelia* IgM titer did not discriminate between the positive and negative assays (Table 2 and Figure 2A; $p = 0.70$). Conversely, the anti-*Borrelia* IgG titer (AU/mL) differed significantly when comparing the positive and negative assays (Table 2 and Figure 2B; $p < 0.0001$). Surprisingly, a high individual titer in the EIA (> 100 AU/mL for IgG and > 2 for IgM index) was not discriminant per se, and so was not reliable for diagnosis unless confirmed in the Western blot assay (Table 2).

Due to the diagnostic importance of the confirmatory test, we next looked at whether the number of lines present on the Western blot was related to the value of the EIA index. The frequency of appearance of each line on the blot was also studied in detail (Figure 3).

As shown in Figures 3A and 3B, the EIA index was correlated within the number of lines on the Western blot for both anti-*Borrelia* IgM and anti-*Borrelia* IgG; the higher the EIA index, the greater the number of lines on the Western blot.

A detailed analysis of the confirmatory Western blots for IgM and IgG (Figure 3C) indicated that the lines were more diverse for IgG than for IgM. Indeed, the Western blot was sometimes positive for the whole panel of anti-*Borrelia* IgGs. The number of lines was lower for anti-*Borrelia* IgMs. The outer surface protein OspC (p25) and the variable major protein-like sequence VlsE proteins were detected on more than 70% of confirmatory IgM and IgG Western blots. In contrast, the p17, p19 and p21 proteins were never detected on the IgM Western blots.

Characteristic of the Patients Screened for Lyme Disease

As stated above, the overall seroprevalence along patients presenting with generalized musculoskeletal pain, neurologic diseases or presumed Lyme borreliosis was 6.0%. We assessed a number of variables (sex, age, and the initial clinical diagnosis) among the 125 patients with a positive Western blot assay (Table 3). Surprisingly, we found a significant sex difference in the distribution of IgG- and IgM-

Table 3: Demographic characteristics of the 125 patients with confirmed positive anti-*Borrelia* antibody assay.

	Positive confirmed anti-*Borrelia* IgGs	Positive confirmed anti-*Borrelia* IgMs	Positive confirmed anti-*Borrelia* IgGs and IgMs
	N=53	N=62	N=10
Sex			
Male (%)	33 (62.3)	18 (29.0)	5 (50.0)
Female (%)	20 (37.7)	44 (71.0)	5 (50.0)
Age (mean years ± SD)	54.4 ± 16.8	46.0 ± 13.5	51.9 ± 21.7
Confirmed anti-*Borrelia* pattern			
Lyme disease as the diagnosis (N=16)	8 (15.1)	2 (3.2)	6 (60.0)
Differential diagnosis (N=37)	18 (34.0)	19 (30.6)	0 (0.0)
No diagnosis (N=72)	27 (50.9)	41 (66.2)	4 (40.0)
Treated for Lyme disease (N=31)	15 (28.3)	9 (14.5)	7 (70.0)

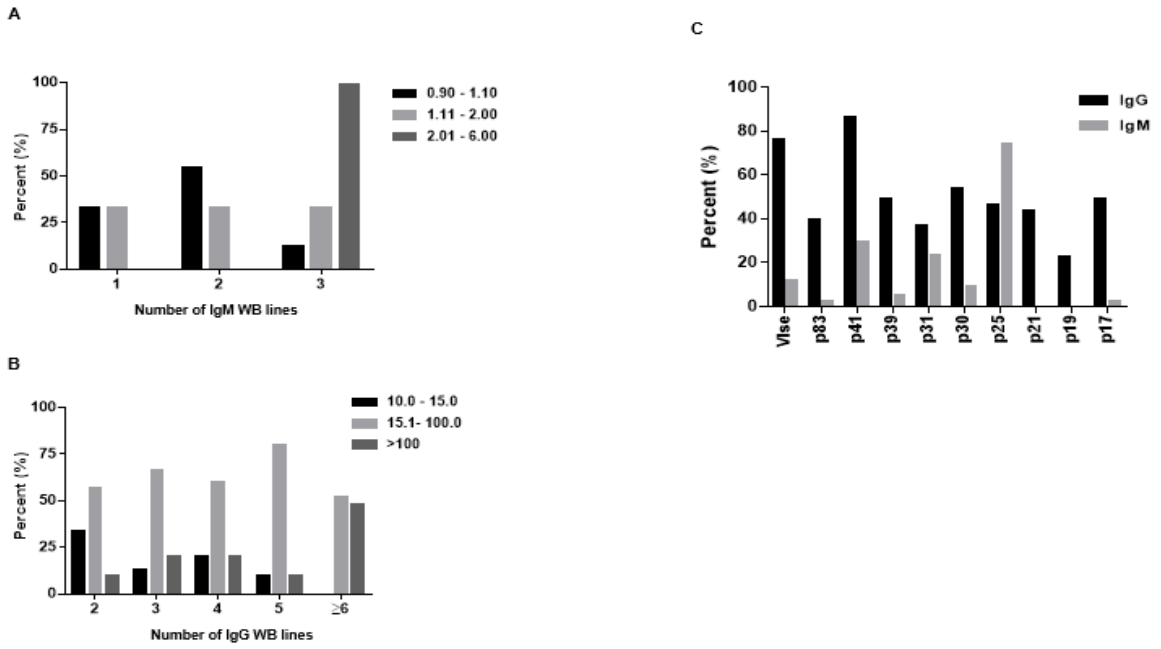

Figure 3: The number of positive lines in the confirmatory Western blot assay, as a function of the value in the anti-*Borrelia* IgM EIA (A) and anti-*Borrelia* IgG EIA (B). The frequency of occurrence of antibodies against specific proteins in the confirmatory Western blot assays for anti-*Borrelia* IgGs and IgMs (C).

positive serologies. Anti-*Borrelia* IgMs were predominant in women; conversely, anti-*Borrelia* IgGs were predominant in men ($p < 0.001$). As expected, there was a significant difference in serologic status as a function of age; patients who were positive for anti-*Borrelia* IgMs alone were typically younger than those who were positive for anti-*Borrelia* IgGs alone ($p < 0.001$).

We next analyzed the serologic status as a function of the patients' screening pattern (Table 3). When considering patients who were positive for anti-*Borrelia* IgGs alone, 27 (50.9%) had nonspecific clinical manifestations. Only 8 of these 53 patients (15.1%) had clinical manifestations specifically related to Lyme disease. The results were even less specific for patients who were positive for anti-*Borrelia* IgMs alone: 41 (66.2%) had nonspecific clinical manifestations, and only 3.2% (2 of the 62 cases) had specific manifestations of Lyme disease. Given that few patients were positive for anti-*Borrelia* IgGs and IgMs, a comparison with the other groups was problematic. In summary, physicians diagnosed Lyme borreliosis in only 16 patients (mean age: 36.7 years;), however, 31 patients weFre treated for Lyme borreliosis. Only 37 of the 125 (29.6%) patients positive for IgGs and/or IgMs in the Western blot assay were given a differential diagnosis: a neurological disease in 23 cases (n=11 for multiple sclerosis, and n=11 for stroke, notably), another internal medical condition in 9 cases, and another infectious disease in 5 cases.

Discussion

Over a 2-year period, we retrospectively analyzed data on specific anti-*Borrelia* antibodies in a population of patients consulting for pain compatible with a diagnosis of disseminated Lyme disease. Of the 2088 individuals tested, 125 (6.0%) were positive for serum anti-*Borrelia* IgGs and/or IgMs. The Sentinel disease monitoring network estimated that the mean annual incidence of Lyme borreliosis in metropolitan France between 2009 and 2017 was 53 per 100,000 inhabitants [2]. The incidence reported for our study area in northern

France for the same period (14 to 23 per 100,000 inhabitants) was below the national average [2]. However, relating the anti-*Borrelia* seroprevalence with the average incidence of Lyme borreliosis is problematic. In fact, serologic assays for anti-*Borrelia* antibodies are very frequently prescribed as part of the diagnostic work-up for polymorphic syndrome. The assays' low positive predictive value decreases their utility.

The recent increase in prescriptions of serologic assays for Lyme borreliosis (whether indicated or not) complicates the interpretation of samples that are positive for anti-*Borrelia* IgGs alone or anti-*Borrelia* IgMs alone.

The broader prescription of anti-*Borrelia* serologic assays leads to the detection of a large number of samples that are positive for anti-*Borrelia* IgGs alone or anti-*Borrelia* IgMs alone, as observed in the present study. We identified several specific profiles. Our results showed that patients with anti-*Borrelia* IgGs alone are predominantly male and tend to be older (Table 3); this probably corresponds to true seroprevalence, which is known to be harmless per se. These patients have probably seroconverted and maintained their immunity over time. The profile of patients with anti-*Borrelia* IgMs alone was totally different, and primarily concerned younger women. These patients may be undergoing an acute infection or they may be carrying nonspecific or cross-reactive antibodies. Our results evidences a clear majority of the population with generalized musculoskeletal pain with a IgM positive serology in this situation. This raises the question of whether it is worth screening for anti-*Borrelia* IgM in the context of long-term infections. Indeed, several studies have found that IgM testing has no added value in patients with a chronic infection [9]. A anti-*Borrelia* serology of control should be proposed 10-15 days after the first screening; if the result does not change, the serologic profile should be interpreted with great caution. Furthermore, we do not have an explanation for the significant difference between men and women observed here; this topic would have to be investigated in a larger cohort.

We also found that the lack of a differential diagnosis leads to the diagnosis of and then treatment for Lyme borreliosis.

Our population was mainly composed of inpatients, with a high proportion of differential diagnoses (mainly for neurologic diseases); this may not be representative of the general population and the indication for serologic testing. Although Lyme borreliosis was diagnosed in only 6.0% of cases, 12% of our out study population had been treated for this disease; it might be that the physician corrected their diagnosis a *posteriori* after the patient had failed to improve.

In this study, we found that the prescription of serologic assays for Lyme disease was prompted by a broad range of non-specific clinical manifestations (mainly neurologic symptoms). In light of the observed seroprevalence, the high proportion of false positives, and the low proportion of true positives, we consider that a serologic assay should not be prescribed as a first-line diagnostic test for patients with generalized musculoskeletal pain. Interpretation of results should be done within a multidisciplinary team.

Funding Information

Acknowledgements

We are grateful to Mathieu Maillard for expert technical assistance.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Sykes RA, Makiello P. An estimate of Lyme borreliosis incidence in Western Europe. J Public Health Oxf Engl. 2017; 39: 74-81.
2. Figoni J, Chirouze C, Hansmann Y, Lemogne C, Hentgen V, Saunier A, et al. Endorsed by scientific societies. Lyme borreliosis and other tick-borne diseases. Guidelines from the French Scientific Societies (I): prevention, epidemiology, diagnosis. Med Mal Infect. 2019; 49: 318-334.
3. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease-a tick-borne spirochetosis? Science. 1982; 216: 1317-1319.
4. Nelson CA, Saha S, Kugeler KJ, Delorey MJ, Shankar MB, Hinckley AF, Mead PS. Incidence of Clinician-Diagnosed Lyme Disease, United States, 2005-2010. Emerg Infect Dis. 2015; 21: 1625-1631.
5. Dessau RB, van Dam AP, Fingerle V, Gray J, Hovius JW, Hunfeld KP, et al. To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2018; 24: 118-124.
6. Raffetin A, Saunier A, Bouiller K, Caraux-Paz P, Eldin C, Gallien S, et al. Unconventional diagnostic tests for Lyme borreliosis: a systematic review. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol. 2019
7. Jaulhac B, Saunier A, Caumes E, Bouiller K, Gehanno JF, Rabaud C, et al. Endorsed by scientific societies. Lyme borreliosis and other tick-borne diseases. Guidelines from the French scientific societies (II). Biological diagnosis, treatment, persistent symptoms after documented or suspected Lyme borreliosis. Med Mal Infect. 2019; 49: 335-346.
8. Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease. Clin Infect Dis Off Publ Infect Dis Soc Am. 2001; 33: 780-785.
9. Ang CW, Brandenburg AH, van Burgel ND, Bijlmer HA, Herremans T, Stelma F, et al. Dutch Working Group on Diagnosis of Lyme Borreliosis. A Dutch nationwide evaluation of serological assays for detection of Borrelia antibodies in clinically well-defined patients. Diagn Microbiol Infect Dis. 2015; 83: 222-228.
10. Eldin C, Raffetin A, Bouiller K, Hansmann Y, Roblot F, Raoult D, Parola P. Review of European and American guidelines for the diagnosis of Lyme borreliosis. Med Mal Infect. 2019; 49: 121-132.
11. Ranque-Garnier S, Eldin C, Sault C, Raoult D, Donnet A. Management of patients presenting with generalized musculoskeletal pain and a suspicion of Lyme disease. Med Mal Infect. 2019; 49: 157-166.