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Article

Clinical, Laboratory, Imaging and Electrocardiographic Differences between Patients with Lyme Disease and Patients with Lyme Disease and *B. divergens* Antibodies

María Folgueras ^{1,2}, Luis Miguel González ³, Julio Collazos ⁴, Mercedes Rodríguez-Pérez ⁵, Laura Pérez- Is ², Javier Diaz-Arias ², Maria Meana ⁶, Belen Revuelta ³, Estrella Montero ^{3,*} and Víctor Asensi ^{1,2}

¹ Infectious Diseases Unit, Hospital Universitario Central de Asturias, Oviedo University School of Medicine, Avda Roma s/n 33011 Oviedo, Spain; mariafolgueras@gmail.com (M.F.); vasensia@gmail.com (V.A.)

² Microbiology and Infectious Diseases Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA) Oviedo Avda Roma s/n 33011 Oviedo, Spain; laura.perzis@gmail.com (L.P.I.); javidiarias@gmail.com (J.D.A.)

³ Parasitology Reference and Research Laboratory, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, 28220, Madrid, Spain.; lmgonzal@isciii.es (L.M.G.); belen.revuelta@isciii.es (B.R.); estrella.montero@isciii.es (E.M.)

⁴ Infectious Diseases Unit, Hospital de Galdácano, Vizcaya Labeaga Auzoa, 48960 Galdácano, Vizcaya, Spain; jcollazos@telefonica.net (J.C.)

⁵ Microbiology Service, Hospital Universitario Central de Asturias, Oviedo University School of Medicine, Avda Roma s/n 33011 Oviedo, Spain; mercedes.rodriguezp@sespa.es (M.R.)

⁶ Internal Medicine Service, Hospital Vital Álvarez-Buylla, Vistalegre, 2, 33611 Mieres, Asturias, Spain; mariameana@gmail.com (M.M.)

* Correspondence: estrella.montero@isciii.es

Abstract: Clinical and diagnostic tests differences between adult patients monoinfected with *Borrelia burgdorferi* sensu lato (s.l.) and patients infected with *B. burgdorferi* s.l. who also had IgG antigens against *Babesia divergens* have not been reported so far. Both Lyme disease caused by *B. burgdorferi* and babesiosis caused by *B. divergens*, endemic in Northwestern Spain, are transmitted by Ixodes tick bites. Clinical, laboratory and other diagnostic tests characteristics (imaging, electrocardiographic-ECG) of 120 residents of Northwestern Spain with *B. burgdorferi* s.l. infection, diagnosed during 2015-2017, of whom 47 (39.2%) had *B. divergens* IgG antibodies, were retrospectively compared. Cardiorespiratory symptoms were reported in 9/47 (19.2%) patients with Lyme disease and *B. divergens* IgG antibodies compared to 4/73 (5.5%) patients with *B. burgdorferi* s.l. monoinfection ($P=0.02$). Dyspnea was recorded in 4/47 (8.5%) patients with Lyme disease and *B. divergens* IgG antibodies compared to 1/73 (1.4%) monoinfected patients ($P=0.07$). In addition ECG atrioventricular (AV) block was detected in 5/47 (15.6%) patients with Lyme disease and *B. divergens* IgG antibodies compared to 1/73 (2.6%) monoinfected individuals ($P=0.09$). No other clinical, laboratory or other tests differences were observed between doubly infected and monoinfected patients. We conclude that patients with Lyme disease and *B. divergens* IgG antibodies had more frequently cardiorespiratory symptoms, mostly dyspnea, compared to monoinfected individuals. These symptoms were unrelated to anemia. ECG AV block perhaps induced by summative myocardial damage due to both infections might play some role in the cardiorespiratory dysfunction.

Keywords: *Babesia divergens*; babesiosis; *Borrelia burgdorferi* sensu lato; cardiorespiratory symptoms; electrocardiographic (ECG) atrioventricular (AV) block; Lyme disease; myocardial damage

1. Introduction

In recent years, tick-borne diseases (TBDs) seem to have increased substantially worldwide [1,2]. Several tick-borne bacterial, viral, and protozoan pathogens cause infection in humans and animals. Among these infections are Lyme disease or borreliosis, but also other less common diseases such as babesiosis, the prevention and treatment of which is hampered by suboptimal diagnostics [1–3].

Asturias (Northwestern Spain) has a mild humid weather and it is covered by 210 Kha of natural **forest**, extending over 61% of its **land area**. **In these forested areas**, ticks and wild roe and fallow deer abound, especially in its most mountainous east and southwest parts. In these areas *Ixodes ricinus* carriers of *Borrelia burgdorferi* sensu lato (s.l.) have been frequently observed [4,5]. These circumstances explain the high seroprevalence of Lyme disease in Asturias, higher than in other regions of Spain, reaching to 5.1% in healthy blood donors of this region [6]. Human babesiosis has also been reported in Asturias [7–9]

Although it affects primarily cattle in Europe, *Babesia divergens*, a parasite also transmitted by *I. ricinus*, can infect humans as well causing babesiosis. *B. divergens*-like DNA sequences have also been reported in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), reindeer (*Rangifer tarandus*) and fallow deer [10,11] However, there is no reported evidence that *Babesia* isolates from deer are infective to humans or cattle so far [12].

Coinfection with *B. burgdorferi* and *Babesia microti* is relatively common in northwestern and upper midwestwestern United States. Approximately 10% of patients with Lyme disease in southern New England are co-infected with babesiosis in locations where both diseases are zoonotic [13]. In Europe *B. microti* and *B. divergens* positive antibodies were observed in 16.3% of Swedish patients seropositive for *B. burgdorferi* [14]. Very recently an Irish longitudinal TBDs study, reported 30% of patients with positive antibody responses to tick-borne pathogens (TBP) including *B. burgdorferi* species and *B. microti*. These patients were treated with combination antibiotics that effectively relieved TBI symptoms with good patient tolerance [15].

Since 2011 two cases of severe infection by *B. divergens* have been reported in Asturias, probably the iceberg “tip” of an undetected infected population [8,9]. Of note, we have recently detected a *B. divergens* seroprevalence rate of 39.2% in 120 patients with Lyme disease in a retrospective study in Asturias (2015-2017) confirming that persons with Babesia-positive antibodies exceed the number of clinically diagnosed human babesiosis cases, so far. This result also warned of possible consecutive or simultaneous undetected co-infections in *B. burgdorferi* s.l.-infected patients [16].

The number of symptoms and duration of illness in American patients with concurrent Lyme disease and babesiosis due to *B. microti* are higher than in patients with either infection alone [13,17–19].

Illness characteristics of patients infected with both *B. burgdorferi* s.l. and *B. divergens* pathogens have not been reported so far. Diagnostic tools on when to suspect and how to manage babesiosis in patients with Lyme disease are lacking as well as how to distinguish co-infections from mono-infections or uninfected patients which could affect the course and severity of the disease [15,20].

To shed more light on these questions, we designed a retrospective study to compare clinical, laboratory and other studies – imaging and electrocardiographic (ECG) – characteristics of the aforementioned Asturian adult patients with previously confirmed Lyme disease and 39.2 % of them with confirmed IgG antibodies against *B. divergens* [16].

2. Material and Methods

2.1. Patients and Study Design

This retrospective study included 120 patients ≥18 years old residing in Asturias (Northwestern Spain) with an IgG positive serology for Lyme disease from 2015 through 2017 assessed at the Microbiology Service of the Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.

Detection of *B. burgdorferi* s.l. antibodies was done using an automated qualitative test (Vidas, BioMerieux, Madrid, Spain) and by immunoblot (Borrelia IgG IgM EcoLine, Sekisui Diagnostics, Russelsheim, Germany) [6,16]. Patients’ serum samples positive for Lyme disease were stored at -

80°C at the Microbiology Service of the HUCA for further studies. These patients had been followed at the Infectious Diseases, Neurology, Rheumatology and/or Dermatology Services of the HUCA or other regional affiliated hospitals because *B. burgdorferi* serology assessment is centralized at the HUCA at regional level. Patients' electronic medical records were searched and their demographic, epidemiological, including Lyme disease-predisposing factors, clinical, laboratory, imaging, ECG and other data were collected.

Early localized Lyme disease included patients with erythema migrans and positive *B. burgdorferi* s.l. IgG and/or IgM serology. This stage occurred within 1-28 days following the tick bite. Early disseminated Lyme disease was made of patients with multiple erythema migrans, early neuroborreliosis, and carditis. This stage developed 3-12 weeks after the initial infection. Later Lyme disease included patients with arthritis, acrodermatitis chronica atrophicans and late neuroborreliosis. This stage developed months or years after the initial infection. Those with confirmed Lyme arthritis had asymmetrical, monoarticular or oligoarticular arthritis, a synovial fluid with 10,000-25,000 cell/mm³ and a positive anti-Borrelia IgG antibody detection and positive Borrelia IgG and/or IgM serology. Other patients with rheumatological symptoms (arthralgias, myalgias) were also included as rheumatological Lyme if they had positive Borrelia IgG and/or IgM serology and no other cause to explain their symptoms. Patients with Lyme carditis had mostly syncope, chest pain or dysnea, positive Borrelia IgG and/or IgM serology and no other cause to explain their heart symptoms. Patients with confirmed neuroborreliosis had compatible clinical symptoms and/or signs (mostly limbs paresthesia/paresia, gait disturbance, cranial neuritis and headache), cerebrospinal fluid (CSF) pleocytosis and CSF positive Borrelia IgG serology. Late Lyme disease occurred months or years after the initial infection. The typical symptoms consisted of neurological and rheumatological involvement. Past Borrelia infection included asymptomatic patients with positive Borrelia IgG serology [21,22]. In a subsequent study, stored serum samples seropositive for *B. burgdorferi* s.l. were used again to detect IgG antibodies against *B. divergens* by an in-house indirect fluorescent assay (IFA) and by Western Blot at the Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain [16].

2.2. Statistical analysis

The continuous variables did not follow a Gaussian distribution, according to the Kolmogorov-Smirnov test and, therefore, non-parametric tests were used for analysis. The values are reported as median (IQ range) or as percentage for continuous or categorical variables, respectively.

The group of patients infected with *B. burgdorferi* s.l. who also had *B. divergens* IgG antibodies and the group of patients only infected with *B. burgdorferi* s.l. were compared with the Mann-Whitney U test for continuous and the chi-square test and the Fisher's exact test, when appropriate, for categorical variables. A stepwise logistic regression analysis model was constructed to identify the factors independently associated with the diagnosis of *B. burgdorferi* s.l. plus positive *B. divergens* IgG antibodies. A P value <0.05 for a two-tailed test was considered statistically significant. Calculations were carried out with the statistical software SPSS v. 25 (IBM Corp., Armonk, New York, USA).

3. Results

A total of 120 patients, 73 monoinfected with *B. burgdorferi* s.l. and 47 infected with *B. burgdorferi* who also had *B. divergens* IgG antibodies were included in the study [16]. The median age of the series was 57.5 years (IQ range 43.8-72.5) and 80 patients (66.7%) were men.

Table 1 shows the demographic, epidemiologic and Lyme disease predisposing factors of *B. burgdorferi* s.l. monoinfected patients and patients infected with *B. burgdorferi* s.l. who also had *B. divergens* IgG antibodies. It can be appreciated that 65.8% developed tick-bites risky outdoor activities, mostly as a hobby, 28.3% had non-professional animal contact and only 10.8% were farmers or cattle breeders. Interestingly only 38.3 % of the Lyme disease patients recalled a recent tick bite. There were no patients with asplenia and only 4 (0.03%) had a known immunodepression. No differences between both groups of infected patients regarding demographic, epidemiologic and Lyme disease predisposing factors were observed.

Table 1. Demography, epidemiology, and predisposing factors.

		Monoinfected (n=73)	Infected and with <i>B. divergens</i> IgG antibodies (n=47)	P value
<u>Demography & epidemiology</u>				
- Gender	Male	47 (64.4%)	34 (72.3%)	0.4
	Female	26 (35.6%)	13 (27.7%)	
- Age years (n=119)		58.0 (42.8-73.6)	56.4 (45.5-71.8)	0.8
- Occupation	Farmer	4 (8.5%)	4 (12.1%)	0.2
	Breeder	3 (6.4%)	2 (6.1%)	
	Open air activity	0 (0%)	3 (9.1%)	
	Other	40 (85.1%)	24 (72.7%)	
- Tick bite identified	Yes	17 (24.6%)	9 (20.9%)	0.7
	No	52 (75.4%)	34 (79.1%)	
- Tick removal	Yes	12 (17.4%)	8 (18.6%)	0.9
	No	57 (82.6%)	35 (81.4%)	
<u>Predisposing factors</u>				
	Yes	66 (90.4%)	42 (93.3%)	0.7
	No	7 (9.6%)	3 (6.7%)	
- Transfusion	Yes	0 (0%)	0 (0%)	-
	No	69 (100%)	45 (100%)	
- Asplenia	Yes	0 (0%)	0 (0%)	-
	No	69 (100%)	45 (100%)	
- Immunosuppression	Yes	2 (2.9%)	2 (4.4%)	0.6
	No	68 (97.1%)	43 (95.6%)	
- Age >50 years	Yes	41 (57.7%)	27 (60.0%)	0.9
	No	30 (42.3%)	18 (40.0%)	
- Outdoor hobbies	Yes	45 (72.6%)	34 (82.9%)	0.2
	No	17 (27.4%)	7 (17.1%)	
- Non-professional animal contact	Yes	18 (29.0%)	16 (39.0%)	0.3
	No	44 (71.0%)	25 (61.0%)	

Values are expressed as median (IQ range) or %.

The clinical features and complications of the patients are reported in Table 2. Overall, 48.3% had neurological symptoms, raising to 53.2% in *B. burgdorferi* s.l. infected patients and with *B.*

divergens IgG antibodies, 14/120 (11.7%) had unilateral or bilateral facial paralysis and 25.8% cutaneous symptoms with erythema migrans reported in 22/120 (18.3%). On the other hand 32.5% had osteomuscular symptoms, mostly arthralgias (27.5%), and 20.8% had constitutional symptoms, mostly fever (11.7%). There were no differences between both groups of infected patients regarding these clinical manifestations. On the other hand 13/120 (10.8%) had cardiorespiratory symptoms, with dyspnea as the most frequent (5/120, 4.12%). Of note, *B. burgdorferi* s.l. infected patients and with *B. divergens* IgG antibodies had more frequently cardiorespiratory symptoms compared to *B. burgdorferi* s.l. monoinfected patients (9/47 [19.1%] vs. 4/73 [5.65%], $P=0.02$) especially dyspnea (4/47 [8.5%] vs. 1/73 [1.4%], $P=0.07$). No differences in the physical exam between both groups of infected individuals were detected. No differences regarding type of Lyme disease, its complications or presence of an alternative diagnosis between both groups of infected patients were observed.

Table 2. Clinical features and complications.

		Monoinfected (n=73)	Infected and with <i>B. divergens</i> IgG antibodies (n=47)	P value
<u>SYMPTOMS</u>				
- Duration of symptoms	days (n=92)	7.0 (1.0-45.0)	15.00 (6.00-105.0)	0.11
<u>Constitutional symptoms</u>	Yes	15 (21.1%)	10 (22.2%)	0.9
	No	56 (78.9%)	35 (77.8%)	
- Fever	Yes	9 (12.7)	5 (11.1%)	0.8
	No	62 (87.3%)	40 (88.9%)	
- Asthenia	Yes	8 (11.3%)	6 (13.3%)	0.7
	No	63 (88.7%)	39 (86.7%)	
- Anorexia	Yes	2 (2.8%)	0 (0%)	0.5
	No	69 (97.2%)	45 (100%)	
- Weight loss	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
<u>Osteomuscular symptoms</u>	Yes	22 (31.0%)	17 (37.8%)	0.5
	No	49 (69.0%)	28 (62.2%)	
- Arthralgias	Yes	19 (26.8%)	14 (31.1%)	0.6
	No	52 (73.2%)	31 (68.9%)	
- Arthritis	Yes	12 (16.9%)	5 (11.1%)	0.4
	No	59 (83.1%)	40 (88.9%)	
- Myalgias	Yes	7 (9.9%)	9 (20.0%)	0.12
	No	64 (90.1%)	36 (80.0%)	
<u>Digestive symptoms</u>	Yes	3 (4.2%)	0 (0%)	0.3
	No	68 (95.8%)	45 (100%)	
- Abdominal pain	Yes	2 (2.8%)	0 (0%)	0.5
	No	69 (97.2%)	45 (100%)	
- Nausea	Yes	0 (0%)	0 (0%)	-

	No	71 (100%)	45 (100%)	
- Vomiting	Yes	1 (1.4%)	0 (0%)	1
	No	70 (98.6%)	45 (100%)	
- Diarrhea	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
<u>Cardiorespiratory symptoms</u>	Yes	4 (5.6%)	9 (20.0%)	0.02
	No	67 (94.4%)	36 (80.0%)	
- Syncope	Yes	1 (1.4%)	2 (4.4%)	0.6
	No	70 (98.6%)	43 (95.6%)	
- Chest pain	Yes	2 (2.8%)	3 (6.7%)	0.4
	No	69 (97.2%)	42 (93.3%)	
- Dyspnea	Yes	1 (1.4%)	4 (8.9%)	0.07
	No	70 (98.6%)	41 (91.1%)	
- Palpitations	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
<u>Neurologic symptoms</u>	Yes	33 (46.5%)	25 (55.6%)	0.3
	No	38 (53.5%)	20 (44.4%)	
- Loss of strength	Yes	11 (15.5%)	9 (20.0%)	0.5
	No	60 (84.5%)	36 (80.0%)	
- Gait disturbance	Yes	8 (11.3%)	5 (11.1%)	1
	No	63 (88.7%)	40 (88.9%)	
- Cranial nerve involvement	Yes	7 (9.9%)	3 (6.7%)	0.7
	No	64 (90.1%)	42 (93.3%)	
- Paresthesia	Yes	8 (11.3%)	7 (15.6%)	0.5
	No	63 (88.7%)	38 (84.4%)	
- Dizziness	Yes	3 (4.3%)	2 (4.4%)	1
	No	67 (95.7%)	43 (95.6%)	
- Headache	Yes	13 (18.3%)	6 (13.3%)	0.5
	No	58 (81.7%)	39 (86.7%)	
- Hyperesthesia	Yes	0 (0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
- Other symptoms	Yes	9 (12.7%)	4 (8.9%)	0.5
	No	62 (87.3%)	41 (91.1%)	
<u>Ophthalmologic symptoms</u>	Yes	3 (4.2%)	1 (2.2%)	1
	No	68 (95.8%)	44 (97.8%)	
- Photophobia	Yes	2 (2.8%)	1 (2.2%)	1
	No	69 (97.2%)	44 (97.8%)	

<u>Cutaneous symptoms</u>	Yes	18 (25.4%)	13 (28.9%)	0.7
	No	53 (74.6%)	32 (71.1%)	
- Erythema migrans	Yes	14 (19.7%)	8 (18.2%)	0.8
	No	57 (80.3%)	36 (81.8%)	
- Rash	Yes	5 (7.0%)	2 (4.4%)	0.7
	No	66 (93.0%)	43 (95.6%)	
- Other cutaneous involvement	Yes	3 (4.2%)	5 (11.1%)	0.3
	No	68 (95.8%)	40 (88.9%)	
<u>Other symptoms</u>	Yes	9 (12.7%)	1 (2.2%)	0.09
	No	62 (87.3%)	44 (97.8%)	
<u>PHYSICAL EXAM</u>				
<u>General</u>	Yes	2 (2.8%)	2 (4.4%)	0.6
	No	69 (97.2%)	43 (95.6%)	
- Jaundice	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Pharyngeal erythema	Yes	0 (0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
- Lymphadenopathy	Yes	2 (2.8%)	2 (4.4%)	0.6
	No	69 (97.2%)	43 (95.6%)	
- Hepatomegaly	Yes	0 (0.0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
- Splenomegaly	Yes	0 (0.0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
<u>Neurologic</u>	Yes	17 (23.9%)	11 (24.4%)	0.9
	No	54 (76.1%)	34 (75.6%)	
- Meningeal signs	Yes	2 (2.8%)	0 (0%)	0.5
	No	69 (97.2%)	45 (100%)	
- Nystagmus	Yes	2 (2.8%)	0 (0%)	0.5
	No	69 (97.2%)	45 (100%)	
- Unilateral facial paralysis	Yes	8 (11.3%)	3 (6.7%)	0.5
	No	63 (88.7%)	42 (93.3%)	
- Bilateral facial paralysis	Yes	2 (2.8%)	1 (2.2%)	1
	No	69 (97.2%)	44 (97.8%)	
- Other cranial nerve involvement	Yes	2 (2.8%)	0 (0%)	0.5
	No	69 (97.2%)	45 (100%)	

- Romberg sign	Yes	1 (1.4%)	0 (0%)	1
	No	70 (98.6%)	45 (100%)	
- Babinski sign	Yes	2 (2.8%)	1 (2.2%)	1
	No	69 (97.2%)	44 (97.8%)	
- Muscle weakness	Yes	12 (16.9%)	7 (15.6%)	0.8
	No	59 (83.1%)	38 (84.4%)	
<u>Ophthalmologic</u>	Yes	1 (1.4%)	1 (2.2%)	1
	No	70 (98.6%)	44 (97.8%)	
- Retinal infarction	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Retinal hemorrhage	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Conjunctival hyperemia	Yes	1 (1.4%)	1 (2.2%)	1
	No	70 (98.6%)	44 (97.8%)	
<u>TYPE OF LYME DISEASE</u>				
- Early localized Lyme disease	Yes	22 (31.9%)	14 (31.1%)	0.9
	No	47 (68.1%)	31 (68.9%)	
- Early disseminated Lyme disease	Yes	12 (17.4%)	7 (15.6%)	0.8
	No	57 (82.6%)	38 (84.4%)	
- Late Lyme disease	Yes	3 (4.3%)	3 (6.7%)	0.7
	No	66 (95.7%)	42 (93.3%)	
- Past Borrelia infection	Yes	21 (30.4%)	16 (35.6%)	0.6
	No	48 (69.6%)	29 (64.4%)	
- Neurologic Lyme disease ¹	Yes	12 (17.4%)	7 (15.6%)	0.8
	No	57 (82.6%)	38 (84.4%)	
<u>COMPLICATIONS</u>	Yes	0 (0.0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
- Respiratory distress	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Heart failure	Yes	0 (0.0%)	1 (2.2%)	0.4
	No	71 (100%)	44 (97.8%)	
- Disseminated intravascular coagulation	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Splenic infarct	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	

- Splenic rupture	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
- Other complications	Yes	0 (0%)	0 (0%)	-
	No	71 (100%)	45 (100%)	
ALTERNATIVE DIAGNOSIS ²	Yes	21 (29.6%)	15 (34.1%)	0.6
	No	50 (70.4%)	29 (65.9%)	

Values are expressed as median (IQ range) or %. ¹ Patients with neurologic Lyme disease had compatible symptoms and/or signs and positive *Borrelia* IgG antibodies in cerebrospinal fluid (CSF). ² Alternative diagnosis represents patients with a primary diagnosis with clinical symptoms and/or signs different from Lyme disease.

Table 3 depicts the laboratory, radiologic imaging and EGC studies of the patients. Anemia, or increased levels of lactate dehydrogenase (LDH) or bilirubin, parameters associated with hemolytic anemia, were not observed neither in both group of patients. Normal levels of C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) were observed in both groups. Atrioventricular (AV) block was detected in 5/47 (15.6%) patients with Lyme disease and *B. divergens* IgG antibodies compared to 1/73 (2.7%) monoinfected individuals (P=0.09). No other imaging or laboratory studies differences were observed between both groups of patients.

Table 3. Imaging, electrocardiographic (EGC) and laboratory studies.

		Monoinfected (n=73)	Infected and with B. divergens IgG (n=47)	P value
Chest X-ray	Normal	38 (97.4%)	26 (92.9%)	0.6
	Abnormal	1 (2.6%)	2 (7.1%)	
EGC	Normal	36 (97.3%)	27 (84.4%)	0.09
	AV block	1 (2.7%)	5 (15.6%)	
<u>Laboratory blood determinations</u>				
Total leukocyte counts	cells/ μ L (n=109)	7500.0 (6165.0-9065.0)	7240.0 (5612.5-9520.0)	0.6
Absolute neutrophil counts	cells/ μ L (n=109)	4180.0 (3170.0-5765.0)	4520.0 (2885.0-6552.5)	0.8
Absolute lymphocyte counts	cells/ μ L (n=107)	1940.0 (1460.0-2590.0)	1740.0 (1470.0-2307.5)	0.3
Absolute eosinophil counts	cells/ μ L (n=106)	130.0 (77.5-232.5)	135.0 (72.5-245.0)	0.9
Platelets	per μ L (n=109)	232000 (183000-294000)	236000 (200250-285250)	1
Hemoglobin	gr/dL (n=109)	14.10 (13.40-15.20)	14.40 (13.38-15.23)	0.8
C-reactive protein	mg/L (n=89)	0.350 (0.100-0.925)	0.300 (0.100-1.400)	0.7
Erythrocyte sedimentation rate	mm/h (n=66)	12.0 (4.8-22.0)	11.0 (5.0-29.0)	0.6
Aspartate aminotransferase	U/L (n=37)	24.0 (19.0-36.3)	21.0 (20.0-28.0)	0.9
Alanine aminotransferase	U/L (n=93)	19.0 (15.0-31.0)	23.0 (17.0-28.0)	0.2
Lactate dehydrogenase	U/L (n=39)	208.0 (182.0-237.0)	207.0 (168.8-254.5)	0.9

Total bilirubin	mg/dL (n=73)	0.90 (0.90-0.90)	0.90 (0.90-0.90)	0.3
Alkaline phosphatase	U/L (n=89)	73.0 (59.0-87.0)	62.0 (52.5-83.3)	0.2
Creatinine	mg/dL (n=106)	0.85 (0.74-1.02)	0.80 (0.74-0.93)	0.4

Values are expressed as median (IQ range) or %.

The microbiological diagnostic procedures are described in Table 4. Positive *B. burgdorferi* IgG serology tests was observed in monoinfected and in those with *B. divergens* IgG by definition. In addition *B. burgdorferi* IgM serology was positive in > 55% of all the patients included in the study. Positive antibodies against *B. divergens* assessed by indirect immunofluorescence assay (IFI) were identified in 47 serum samples from patients, and 40 serum of the total were confirmed by Western-blot (40/47-85.1%) using *B. divergens* extract proteins. Moreover, IgG antibodies against the major surface antigen (Bd37) of *B. divergens* were found in 19 /47 (47.5%) of the samples [16,23,24].

Table 4. Microbiological studies.

		Monoinfected (n=73)	Infected and with B. divergens IgG (n=47)	P value
<i>Borrelia burgdorferi</i> IgM serology	Positive	43 (58.9%)	27 (57.4%)	0.9
	Negative	30 (41.1%)	20 (42.6%)	
<i>Borrelia burgdorferi</i> IgG serology	Positive	73 (100%)	47 (100%)	-
	Negative	0 (0%)	0 (0%)	
<i>Borrelia burgdorferi</i> immunoblot	Positive	62 (88.6%)	39 (95.1%)	0.5
	Negative	1 (1.4%)	0 (0%)	
	Doubtful	7 (10.0%)	2 (4.9%)	
<i>Babesia divergens</i> indirect immunofluorescence assay	Positive	0 (0%)	47 (100%)	<0.0001
	Negative	73 (100%)	0 (0%)	
<i>Babesia divergens</i> protein extract ¹	Positive	0 (0%)	40 (85.1%)	<0.0001
	Negative	73 (100%)	7 (14.9%)	
Bd37 recombinant protein ¹	Positive	0 (0%)	15 (32 %%)	<0.0001
	Negative	73 (100%)	32 (68%)	

¹ *B. divergens* protein extracts and the purified Glutathione S-Transferase-tagged Bd37 recombinant protein (GST-rBD37) were used as target substrates for *B. divergens* Western-blot assays.

Table 5 shows the treatment administered to the patients. The most commonly administered antibiotic was doxycycline and there were no significant differences between both groups of patients in any of the regimens administered.

Table 5. Antibiotic treatment.

		Monoinfected (n=73)	Infected and with <i>B. divergens</i> IgG (n=47)	P value
Doxycycline	Yes	21 (31.3%)	19 (44.2%)	0.17
	No	46 (68.7%)	24 (55.8%)	
Doxycycline dosage	mg/day (n=39)	200 (200-200)	200 (200-200)	0.3
Amoxicillin	Yes	5 (7.5%)	5 (11.4%)	0.5
	No	62 (92.5%)	39 (88.6%)	
Amoxicillin dosage	mg/day (n=11)	1500 (650-2625)	1500 (938-2625)	1
Ceftriaxone	Yes	10 (14.9%)	7 (15.9%)	9
	No	57 (85.1%)	37 (84.1%)	
Ceftriaxone dosage	mg/day (n=17)	1500 (1000-2000)	2000 (1000-2000)	0.8
Cefuroxime	Yes	1 (1.5%)	0 (0%)	1
	No	66 (98.5%)	44 (100%)	
Azithromycin	Yes	0 (0%)	1 (2.2%)	0.4
	No	67 (100%)	44 (97.8%)	
Penicillin G	Yes	1 (1.5%)	0 (0%)	1
	No	66 (98.5%)	44 (100%)	
Treatment duration	weeks (n=108)	2.00 (0.00-3.00)	2.00 (0.00-3.00)	0.7

Values are expressed as median (IQ range) or %.

A forward stepwise logistic regression analysis model, including all variables with a P value ≤ 0.1 in the univariate analyses, revealed that the only significantly independent predictive factor for *B. burgdorferi* s.l.- infection of patients with *B. divergens* IgG antibodies was the presence of cardiorespiratory symptoms (OR 4.184, 95% CI 1.205-14.493, P=0.024).

4. Discussion

Borrelia burgdorferi s.l. infected patients who also had *B. divergens* IgG antibodies had more frequently cardiorespiratory symptoms, mostly dyspnea, compared to *B. burgdorferi* s.l. monoinfected individuals. These cardiorespiratory symptoms were unrelated to anemia that both groups of patients did not have, and might be enhanced by ECG AV block. This cardiac arrhythmia could be caused by summative myocardial damage induced by both pathogens.

Babesiosis due to *B. divergens* is the most severe among the infections due to all *Babesia* species in humans with a quite high mortality, up to 40% in hospitalized patients, although nowadays it is decreasing [2]. Other *Babesia* species such as *B. microti* have a lower mortality rate reaching 10-20% in hospitalized individuals [25,26]. Mortality attributable to *Babesia* is not due exclusively to the pathogen or the host but also to the interaction between both of them. This interaction explains the severity of some cases, two of them reported from Asturias, one in a young immunocompetent patient, and the lower severity of others such as those reported in the present study [8,9].

Previous reports showed that experimental coinfection by *B. burgdorferi* s.l. and *B. microti* induced increased arthritis severity in mice which correlated with a significant reduction of expression of the IL-10 and IL-13 cytokines [27]. *B. burgdorferi*-*B. microti* coinfection in humans increased the intensity and duration of the illness and its symptoms in American patients [13,17]. Although the number and duration of babesial symptoms were similar in older and younger of 50

years infected by *B. microti* and babesial-Lyme disease coinfecting subjects, more older adults were admitted to the hospital than younger in other American study cohort [19]. Fatigue, but also headache, nausea, sweating, anorexia, chills, emotional lability and splenomegaly were more frequently reported in *B. burgdorferi*-*B. microti* coinfecting individuals with symptoms' duration lasting up to three months compared to those individuals monoinfected with *B. burgdorferi* s.l. No cardiac abnormalities have been described in these coinfecting American patients, though [13].

To our knowledge this is the first report studying clinical differences, laboratory, imaging and ECG of *B. burgdorferi* s.l.-*B. divergens*-infection in the literature and the second in which *B. divergens* antibodies are detected among *B. burgdorferi*-infected patients [14]. A recent French meta-analysis found eight reports of *B. burgdorferi* s.l.-*Babesia* spp. coinfection in the literature but all of them were due to *B. microti* [28].

Overall, 53.2% of patients with *B. burgdorferi* s.l. and with IgG antibodies against *B. divergens* had minor neurologic symptoms, mostly facial paralysis and muscle weakness. These symptoms seem more related to *B. burgdorferi* s.l. than to *B. divergens* infection. Neurologic symptoms of babesiosis are headache, confusion/delirium, impaired consciousness, ataxia/gait disorder and vision impairment not described by patients with *B. burgdorferi* s.l. and with IgG antibodies against *B. divergens* in our study, except for headache that was reported in 13.3 % of the patients (Table 2) [30]. Only 22.2% of the patients reported constitutional complaints (fever, asthenia, anorexia, weight loss). In addition their clinical course was mild and short unlike the complicated clinical course of those with *B. burgdorferi* s.l.-*B. microti* coinfections [17,19]. A possible explanation for this discordance might be differences in the susceptibility of individual patients to infection.

It is intriguing that patients infected with *B. burgdorferi* s.l. and with IgG antibodies against *B. divergens* had more frequently cardiorespiratory symptoms, mostly dyspnea compared to *B. burgdorferi* s.l. monoinfected patients. These were the only significant differential clinical symptoms between both groups of patients. One possible explanation for the cardiac dysfunction in the group with Lyme disease and *B. divergens* IgG antibodies might be a summative potential myocardial damage of both microorganisms manifested by ECG AV block present in five individuals. Both *B. burgdorferi* s.l. and *B. divergens* might affect AV conduction leading to AV block. *B. burgdorferi* AV block is a well-known complication of Lyme disease [31]. *Babesia bigemina* infection induced changes in cardiac function biomarkers and D-dimer in cattle [32]. *Babesia canis* infection induced cardiac disorders in dogs with 32% of AV block [33]. Two reports showed myocardial damage and serious arrhythmia associated with severe babesiosis in two American patients [34,35]. A recent report showed that 19.6% of hospitalized American patients with acute babesiosis due to *B. microti* developed cardiac complications, mostly atrial fibrillation, heart failure, QT interval prolongation and cardiac ischemia [36]. No AV block I in that study was observed, though. All the patients had high parasitemia and had received antimicrobials (macrolides, quinine) which might have enhanced their heart arrhythmia. Interestingly cardiac complications were not worse in 12 of them with confirmed *B. burgdorferi* s.l.-*B. microti* coinfection.

We assume that *B. divergens* might induce a myocardial damage similar to *B. microti*. Unluckily, neither troponin, creatin kinase (CK), natriuretic peptides (B-type natriuretic peptide [BNT] or N-terminal pro-B-type natriuretic peptide [INT-proBNT]), other cardiac function biomarkers or echocardiograms were done in our study being retrospective. In addition ECG was performed in only 58.4% of our patients.

Thus, the mean weakness of the study is derived of its retrospective design lacking universal ECG, cardiac biomarkers and echocardiograms testing that might provide deeper insight into the mechanism of cardiac complications in the patients infected with Lyme disease and *B. divergens* IgG antibodies. Other weakness of the study is the uncertainty of the timing, simultaneous or sequential, of both infections, by *B. burgdorferi* s.l. and *B. divergens*. Patients could be firstly infected by *B. divergens* to become infected by *B. burgdorferi* subsequently. If this be the case we might be comparing virtually two groups with a *B. burgdorferi* s.l. monoinfection. The fact that even so clinical differences between both groups of infected patients were observed suggests that disparity might be higher in cases of a confirmed simultaneous coinfection.

In summary patients with Lyme disease and *B. divergens* IgG antibodies had a mild clinical course, and did not develop anemia or other analytical abnormalities. Their only, but significant differential symptoms were cardiorespiratory manifested as dyspnea and ECG AV block.

5. Conclusions

Patients with suspected Lyme disease could be screened for babesiosis as well to obtain relevant information on epidemiology and prevalence of both concurrent zoonosis in endemic areas. Detection of sequential and simultaneous infections might also provide useful information to the physician to monitor and treat, with the most effective antimicrobial regimen, patients living in these areas. This concern is especially important in those immunocompromised in whom babesiosis has a more severe evolution and could lead even to a fatal outcome.

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