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María Folgueras , Luis Miguel Gonzalez , Julio Collazos , Mercedes Rodríguez-Pérez , Laura Pérez-Is , Javier Diaz-Arias , María Meana , Belen Revuelta , Estrella Montero * , Víctor Asensi

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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

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Abstract: Clinical and analytical differences between adult patients monoinfected with *Borrelia burgdorferi sensu lato* (s.l.) and patients infected with *B. burgdorferi s.l.* who also had IgG antibodies against *Babesia divergens* have not been reported so far. Both Lyme disease caused by *B. burgdorferi* and babesiosis caused by *B. divergens*, endemic in Asturias, Northwestern Spain, are transmitted by *Ixodes* tick bites. Clinical, laboratory and other diagnostic tests characteristics (imaging, electrocardiographic-ECG) of 120 residents of Asturias with confirmed *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 (P=0.07). In addition ECG atrioventricular (AV) block, mostly 1st degree, was detected in 5/47 (15.6%) patients with Lyme disease and *B. divergens* IgG antibodies compared to 1/73 (2.6%) *B. burgdorferi s.l.* monoinfected (P=0.09). No other clinical, laboratory or other tests differences were observed between *B. burgdorferi* and *B. divergens* IgG antibodies carriers and *B. burgdorferi* monoinfected. We concluded that patients with Lyme disease and *B. divergens* IgG antibodies had more frequent cardiorespiratory symptoms, mainly dyspnea, compared to *B. burgdorferi* monoinfected patients. These symptoms were unrelated to anemia. ECG AV block, perhaps induced by summative myocardial damage due to either infections or *B. divergens* induced lung microcirculation slowing-down, might play a role in the cardiorespiratory dysfunction.

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

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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, some of which induce well-known human diseases. Among these illnesses are Lyme disease or borreliosis, but also other less common diseases such as babesiosis, whose prevention and treatment is hampered by suboptimal diagnosis [1–3].

Ixodes scapularis is the tick vector of both Lyme disease and babesiosis in the United States (US). Coinfection with the main causative agents, *Borrelia burgdorferi* and *Babesia microti*, respectively, is relatively common in Northwestern and Upper Midwestewern US. Approximately 10% of patients with Lyme disease in southern New England are co-infected with babesiosis in areas where both diseases are zoonotic [4].

In Europe *B. burgdorferi*, *B. microti* and *Babesia divergens* are transmitted by *Ixodes ricinus* [5]. Positive antibodies against *B. microti* and *B. divergens* were observed in 16.3% of Swedish patients seropositive for *B. burgdorferi* [6]. Very recently an Irish longitudinal TBDs study, reported 30% of patients with positive antibody responses to tick-borne pathogens (TBPs) including *B. burgdorferi* species and *B. microti.* These patients were treated with combination antibiotics that effectively relieved TBD symptoms with good patient tolerance [7].

Importantly, although atovaquone + azithromycin is the preferred combination to treat babesiosis, with clindamycin + quinine as alternative, the recent combination of tafenoquine, a novel 8-aminoquinoline primaquine analogue + atovaquone has demonstrated to be highly effective against babesiosis, especially in cases with evidence of resistance to the former antimicrobials [8–11].

Asturias (Northwestern Spain) has a mild humid climate 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 *B. burgdorferi* sensu lato (s.l.). have been frequently observed [12,13]. These circumstances explain the high seroprevalence of Lyme disease in Asturias, higher than in other regions of Spain, reaching to 5.1% of positive *B. burgdorferi* s.l. IgG serology in healthy blood donors of this region [14].

Since 2011, human babesiosis has also been reported in Asturias. In fact, the severe babesiosis caused by *B. divergens* in two immunocompetent patients probably pointed to the iceberg tip of an undetected *B. divergens* infected population [15,16].

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 considerably the number of clinical cases of human babesiosis diagnosed so far. This result also presaged possible undetected consecutive or simultaneous *B. divergens* co-infections in *B. burgdorferi* s.l. infected patients [17–19].

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 [4,20–22]. 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.—Means on how to distinguish coinfections from monoinfections or uninfected patients are greatly needed. These diagnostic tools could affect the course and severity of the disease [7,23]. Moreover, differences in *Ixode*s vectors and pathogen prevalence and virulence between the US and Europe make direct comparisons between *B. burgdorferi-B. microti* and *B. burgdorferi-B. divergens co*infections difficult and encourage further research.

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 all with previously confirmed Lyme disease and 39.2 % of them with confirmed serology against *B. divergens* [17].

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Material and Methods

Patients and Study Design

Target Population, Inclusion and Exclusion Criteria

The rationale for this study is the fact that individuals with an IgG positive serology to *B. burgdorferi* s.l. could also have positive serology to *B. divergens*, after exposure to *I. ricinus* bites, the common vector for both Lyme disease and babesiosis. This retrospective study included all the patients ≥18 years old residing in the central part of Asturias (Northwestern Spain) with an IgG positive serology for Lyme disease obtained from 2015 through 2017 assessed at the Microbiology Service of the Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain. 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* s.l. serology assessment is centralized at the HUCA at regional level. Lyme disease was clinically diagnosed by different specialists at HUCA, and confirmed by a positive *B. burgdorferi* s.l. serology (see Lyme disease case definitions below). Patients' electronic medical records were searched and their demographic, epidemiological, including Lyme disease-predisposing factors, clinical, laboratory, imaging, ECG and other data were collected. Patients younger than 18 years were excluded of the study. Overall, 120 patients were included with independence of their Lyme disease stage. The relative small size of our cohort precluded us to stratify the Lyme cases in different clinical stages

Lyme Disease Case Definitions

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/mm^3 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 [24,25].

Laboratory Analysis

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) [14,17]. Patients' serum samples positive for Lyme disease were stored at -80ºC at the Microbiology Service of the HUCA for further studies. 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 [17].

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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 text, 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).

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 four (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 *B. divergens* IgG Antibodies (n=47)**	**P value**
Demography & epidemiology				
- 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%)	
Predisposing factors	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%)	

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- Asplenia	Yes No	0 (0%) 69 (100%)	0 (0%) 45 (100%)	-
- Immunosuppression	Yes No	2 (2.9%) 68 (97.1%)	2 (4.4%) 43 (95.6%)	0.6
- Age >50 years	Yes No	41 (57.7%) 30 (42.3%)	27 (60.0%) 18 (40.0%)	0.9
- Outdoor hobbies	Yes No	45 (72.6%) 17 (27.4%)	34 (82.9%) 7 (17.1%)	0.2
- Non-professional animal contact	Yes No	18 (29.0%) 44 (71.0%)	16 (39.0%) 25 (61.0%)	0.3

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%). *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 *B. divergens* IgG antibodies (n=47)**	**P value**
SYMPTOMS				
- Duration of symptoms	days (n=92)	7.0 (1.0-45.0)	15.00 (6.00-105.0)	0.11
Constitutional symptoms	Yes No	15 (21.1%) 56 (78.9%)	10 (22.2%) 35 (77.8%)	0.9
- Fever	Yes No	9 (12.7) 62 (87.3%)	5 (11.1%) 40 (88.9%)	0.8
- Asthenia	Yes No	8 (11.3%) 63 (88.7%)	6 (13.3%) 39 (86.7%)	0.7
- Anorexia	Yes No	2 (2.8%) 69 (97.2%)	0 (0%) 45 (100%)	0.5
- Weight loss	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
Osteomuscular symptoms	Yes	22 (31.0%)	17 (37.8%)	0.5

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	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%)	
Digestive symptoms	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%)	
Cardiorespiratory symptoms	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%)	
Neurologic symptoms	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%)	

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- Dizziness	Yes No	3 (4.3%) 67 (95.7%)	2 (4.4%) 43 (95.6%)	1
- Headache	Yes No	13 (18.3%) 58 (81.7%)	6 (13.3%) 39 (86.7%)	0.5
- Hyperesthesia	Yes No	0 (0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4
- Other symptoms	Yes No	9 (12.7%) 62 (87.3%)	4 (8.9%) 41 (91.1%)	0.5
Ophthalmologic symptoms	Yes No	3 (4.2%) 68 (95.8%)	1 (2.2%) 44 (97.8%)	1
- Photophobia	Yes No	2 (2.8%) 69 (97.2%)	1 (2.2%) 44 (97.8%)	1
Cutaneous symptoms	Yes No	18 (25.4%) 53 (74.6%)	13 (28.9%) 32 (71.1%)	0.7
- *Erythema migrans*	Yes No	14 (19.7%) 57 (80.3%)	8 (18.2%) 36 (81.8%)	0.8
- Rash	Yes No	5 (7.0%) 66 (93.0%)	2 (4.4%) 43 (95.6%)	0.7
- Other cutaneous involvement	Yes No	3 (4.2%) 68 (95.8%)	5 (11.1%) 40 (88.9%)	0.3
Other symptoms	Yes No	9 (12.7%) 62 (87.3%)	1 (2.2%) 44 (97.8%)	0.09
PHYSICAL EXAM				
General	Yes No	2 (2.8%) 69 (97.2%)	2 (4.4%) 43 (95.6%)	0.6
- Jaundice	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Pharyngeal erythema	Yes No	0 (0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4
- Lymphadenopathy	Yes No	2 (2.8%) 69 (97.2%)	2 (4.4%) 43 (95.6%)	0.6
- Hepatomegaly	Yes No	0 (0.0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4
- Splenomegaly	Yes No	0 (0.0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4

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Neurologic	Yes No	17 (23.9%) 54 (76.1%)	11 (24.4%) 34 (75.6%)	0.9
- Meningeal signs	Yes No	2 (2.8%) 69 (97.2%)	0 (0%) 45 (100%)	0.5
- Nystagmus	Yes No	2 (2.8%) 69 (97.2%)	0 (0%) 45 (100%)	0.5
- Unilateral facial paralysis	Yes No	8 (11.3%) 63 (88.7%)	3 (6.7%) 42 (93.3%)	0.5
- Bilateral facial paralysis	Yes No	2 (2.8%) 69 (97.2%)	1 (2.2%) 44 (97.8%)	1
- Other cranial nerve involvement	Yes No	2 (2.8%) 69 (97.2%)	0 (0%) 45 (100%)	0.5
- Romberg sign	Yes No	1 (1.4%) 70 (98.6%)	0 (0%) 45 (100%)	1
- Babinski sign	Yes No	2 (2.8%) 69 (97.2%)	1 (2.2%) 44 (97.8%)	1
- Muscle weakness	Yes No	12 (16.9%) 59 (83.1%)	7 (15.6%) 38 (84.4%)	0.8
Ophthalmologic	Yes No	1 (1.4%) 70 (98.6%)	1 (2.2%) 44 (97.8%)	1
- Retinal infarction	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Retinal hemorrhage	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Conjunctival hyperemia	Yes No	1 (1.4%) 70 (98.6%)	1 (2.2%) 44 (97.8%)	1
TYPE OF LYME DISEASE				
- Early localized Lyme disease	Yes No	22 (31.9%) 47 (68.1%)	14 (31.1%) 31 (68.9%)	0.9
- Early disseminated Lyme disease	Yes No	12 (17.4%) 57 (82.6%)	7 (15.6%) 38 (84.4%)	0.8
- Late Lyme disease	Yes No	3 (4.3%) 66 (95.7%)	3 (6.7%) 42 (93.3%)	0.7
- Past Borrelia infection	Yes No	21 (30.4%) 48 (69.6%)	16 (35.6%) 29 (64.4%)	0.6

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- Neurologic Lyme disease [1]	Yes No	12 (17.4%) 57 (82.6%)	7 (15.6%) 38 (84.4%)	0.8
COMPLICATIONS	Yes No	0 (0.0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4
- Respiratory distress	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Heart failure	Yes No	0 (0.0%) 71 (100%)	1 (2.2%) 44 (97.8%)	0.4
- Disseminated intravascular coagulation	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Splenic infarct	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Splenic rupture	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
- Other complications	Yes No	0 (0%) 71 (100%)	0 (0%) 45 (100%)	-
ALTERNATIVE DIAGNOSIS [2]	Yes No	21 (29.6%) 50 (70.4%)	15 (34.1%) 29 (65.9%)	0.6

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 bilirrubin, 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). Notably, four patients had a 1st degree AV block and one patient with Lyme disease and *B. divergens* IgG antibodies had a 2nd degree AV block.

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 Abnormal	38 (97.4%) 1 (2.6%)	26 (92.9%) 2 (7.1%)	0.6
EGC	Normal AV block[1]	36 (97.3%) 1 (2.7%)	27 (84.4%) 5 (15.6%)	0.09
Laboratory blood determinations				
Total leukocyte counts	cells/μL (n=109)	7500.0 (6165.0-9065.0)	7240.0 (5612.5-9520.0)	0.6

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Absolute neutrophil counts	cells/µL (n=109)	4180.0 (3170.0-5765.0)	4520.0 (2885.0-6552.5)	0.8
Absolute lymphocyte counts	cells/µL (n=107)	1940.0 (1460.0-2590.0)	1740.0 (1470.0-2307.5)	0.3
Absolute eosinophil counts	cells/µL (n=106)	130.0 (77.5-232.5)	135.0 (72.5-245.0)	0.9
Platelets	per µL (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 %. 4/5 patients had 1st degree AV block and one that had Lyme disease and *B. divergens* IgG positive serology had 2nd degree AV block[1].

No other imaging or laboratory studies differences were observed between both groups of patients.

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 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**
Borrelia burgdorferi IgM serology	Positive	43 (58.9%)	27 (57.4%)	0.9
	Negative	30 (41.1%)	20 (42.6%)	
Borrelia burgdorferi IgG serology	Positive	73 (100%)	47 (100%)	-
	Negative	0 (0%)	0 (0%)	
Borrelia burgdorferi immunoblot	Positive	62 (88.6%)	39 (95.1%)	0.5
	Negative	1 (1.4%)	0 (0%)	
	Doubtful	7 (10.0%)	2 (4.9%)	
Babesia divergens indirect immunofluorescence assay	Positive	0 (0%)	47 (100%)	<0.0001
	Negative	73 (100%)	0 (0%)	

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Babesia divergens protein extract [1]	Positive Negative	0 (0%) 73 (100%)	40 (85.1%) 7 (14.9%)	<0.0001
Bd37 recombinant protein [1]	Positive Negative	0 (0%) 73 (100%)	15 (32 %%) 32 (68%)	<0.0001

[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.

Regarding therapy the most commonly administered antibiotic was doxycycline 200 mg/day, followed by amoxicillin 1.500 mg/day, both for 2 weeks. There were no significant differences between the two groups of patients in any of the antimicrobial regimens administered (Supplementary Table S1).

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).

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. A potential *B. divergens* induced lung microcirculation slowing-down could also play some role in the dyspnea pathogenesis.

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 [26,27]. 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 [15,16].

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 [28]. *B. burgdorferi-B. microti* coinfection in humans increased the intensity and duration of the illness and its symptoms in American patients [4,20]. 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 coinfected subjects, older adults were admitted to the hospital than younger in other American study cohort [22]. Fatigue, but also headache, nausea, sweating, anorexia, chills, emotional lability and splenomegaly were more frequently reported in *B. burgdorferi-B. microti* coinfected 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 coinfected American patients, though [4].

To our knowledge this is the first report studying clinical, laboratory , imaging and ECG differences between patients with confirmed Lyme disses and positive IgG serology to *B. divergens* in the literature and the second in which *B. divergens* antibodies are detected among *B. burgdorferi*-infected patients [6]. 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* [29].

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

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study, except for headache that was reported in 13.3% of the patients (Table 2) [30]. Only 22.2% of the patients reported constitutional complains (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 previously reported [20,22]. A possible explanation for this discordance might be differences in the susceptibility of individual patients to infection including differences in their immune system.

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, one with a 2nd degree AV block. 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 arrhtymia 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 of any degree was observed in that study, though. All the patients had high parasitemia and had received antimicrobials (macrolides, quinine) which might have enhanced their heart arrythmia. 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 natriuretric peptide [BNT] or N-terminal pro-B-type natriuretic peptide ([NT-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. Other possible explanation for the dyspnea reported in patients infected with *B. burgdorferi* s.l. and with IgG antibodies against *B. divergens* could be a dysfunction of the pulmonary microcirculation due to sequestration of parasitized erythrocytes in the host microvasculature. This mechanism has not been documented for *B. divergens*, but it has been reported that sequestration of *Babesia bovis* erythrocytes in the host microvasculature is associated with cerebral and vascular complications of babesiosis in cattle. This effect is mediated by *B. bovis* infected bovine erythrocytes that bind to bovine brain capillary endothelial cells via knobby protrusions on the infected erythrocyte surface [37–39]. Sequestration of *Plasmodium falciparum* infected erythrocytes has also been linked to cerebral malaria [40].

According to our results, *B. divergens* infected erythrocytes adhering to the microvasculature could slow down or block the pulmonary microcirculation, thereby impairing oxygen exchange and causing dyspnea in patients in a similar way.

Our study could help identifying clinically, analytically and by and ECG and other tests patients infected with B. burgdorferi s.l. and those that also carried IgG antibodies against *B. divergens*. Clearance of *B. divergens* is dependent of the innate and adaptative immune system. In most of the immunocompetent individuals, parasitemia rarely persist or relapse even in absence of antiBabesial therapy. The opposite occurs in splenectomized and immunocompromised patients, including those exposed to rituximab, an anti-CD20 monoclonal antibody [41]. In these immunocompromised patients, an early diagnosis might prevent the development of severe, even fatal babesiosis. Atovaquone + azithromycin was the favorite combination to treat babesiosis so far with clindamycin + quinine as useful alternative [8]. However, relapsing babesiosis with identified molecular evidence of resistance to certain antimicrobials such as azithromycin, atovaquone and clindamycin has proved the value of tafenoquine. Tafenoquine in a long oral course perhaps combined to atovaquone might help eradicate *B. microti* infection in immunocompromised mice and humans [9,11,42,43]. No experience with tafenoquine for *B. divergens* infections in humans has been reported so far.

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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. burgdoferi* s.l. and *B. divergens*. Patients could be firstly infected by *B. divergens* to become infected by *B. burgdoferi* subsequently. If this be the case we might be comparing virtually two groups with a *B. burgdoferi* 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.

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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