Significance of Borrelia Infection in Development of Dilated Cardiomypathy (A pilot study)

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Received December 10, 2007; Accepted December 20, 2007.

Key words: Lyme disease – Lyme carditis – Dilated cardiomyopathy

The study was supported by the Grant Agency of the Ministry of Health of the Czech Republic. No. NI/7528-3.

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Abstract: A heart involvement known as Lyme carditis (LC), a consequence of Lyme borreliosis (LB), is relatively rare in contrast to the involvement of skin, joints and nervous system; it accounts for < 4% of all these patients in European countries. However, the diagnosis of the disease belongs to the most difficult challenges. While various forms of AV blocks dominate in the USA as confirmed by the literature, there is a clear predominance of arrhythmias of various incidence in the Czech Republic. The authors of this article focused on the form belonging to the rarest manifestations of LC, namely dilated cardiomyopathy (DCMP). The goal was to elucidate the etiological participation of Borrelia infection in the development of DCMP, which has attracted controversial opinions so far. In total, 33 patients with DCMP were enrolled in the study, 23 males and 10 females, with mean age 57.7 years (range 24–76 years). ELISA NRLB KC 90 method was used in all blood samples for detection of Borrelia infection (BI), Western blot method was used for confirmation, followed by identification of DNA of pathogenic Borreliae using PCR method. Bioptic material was examined by electronmicroscopy with an attempt to detect Spirochaetae in myocardium. 16 patients were excluded from the study owing to the absence of signs of LB. The study group included 17 patients (3 females, 13 males) with mean age 58 years (range 43–76 years), in whom the presence of Bb was proved by identification of DNA of pathogenic Borreliae or by electronmicroscopic detection of Spirochetae in myocardial bioptic sample. The findings obtained during the study confirmed that BI very probably participated in the development of dilated cardiomyopathy. It may be concluded that most of cases were either unapparent forms of LB or insufficiently treated cutaneous forms of this disease.

Introduction

A heart involvement during Lyme borreliosis (LB) manifests in the second stage of the early infection; it is known as Lyme carditis (LC). It was described for the first time in 1890, and subsequently reported by many studies [1–9].

Even though it was found that Spirochetae belong to the etiological agents participating in the development of DCMP, the literary data about participation of Borrelia burgdorferi have been rare so far, despite the marked expansion of LB. Austrian authors were the first to report in 1990 [10] on possible causal relation between Borrelia infection and dilated cardiomyopathy (DCMP). In the same year, other Austrian authors published results pertaining to 81 patients with DCMP, 13 out of them suffered acute myocarditis. Twenty four out of them (29.6%) had antibodies against Bb. Special staining proved Spirochetae in myocardial bioptic samples; Bb was even cultivated in one of the bioptic samples. The isolation of Bb discovered a new subtype of Bb among hitherto isolated Bb, and strengthened the hypothesis about relation between Bb and dilated cardiomyopathy.

Two years later, reports aimed at the evidence of Borrelia infection (BI) in 42 patients with confirmed DCMP were published. In 9 out of them, positivity of serologic examination was found and therapy with cefalosporin was recommended. In 6 out of them EFLV (ejection fraction of left ventricle) was normalized during 6 months, in 2 out of them it was improved, and there was no change in 1 patient. It is necessary to mention that there is no unanimous agreement on Borrelia infection (BI) being an exclusive etiological factor of DCMP [11]. However, BI as etiological agent involved in chronic heart failure was repeatedly proved [12]. Wunderlich's study [13] confirmed BI as the cause of chronic heart failure as well.

Patients and methods

Following requirements were stated for the enrolment in the study:

1. Clinical protocol with objective evidence of DCMP (chest X ray, echocardiogram, coronarography).
2. In all patients, negative history of rheumatic fever, scarlet fever, recurrent tonsillis, negative clinical signs of previous rheumatic fever, absence of inborn or acquired valvular disorders, cardiotoxic alcohol or drug abuse. Ischemic heart disease was ruled out (negative coronarography) and type I diabetes mellitus as well.
3. Informed consent of the patients approved by the Ethical Committee of 1. LF UK and VFN (First Faculty of Medicine and General Teaching Hospital, Prague).

In total, 33 patients with DCMP were enrolled in the study: 23 males and 10 females with mean age 57.7 years, range 24–76 years.

Following examinations were performed: standard 12-lead ECG, dynamic 24-hour Holter electrocardiogram, echocardiogram, ions (serum Na, K, Cl), hormonal activity of the thyroid gland (T3, T4, TSH).

In accordance with the standard procedure within DCMP, myocardial biopsy was performed during coronarography. Bioptic samples were examined at the National Reference Laboratory for Lyme Borreliosis at the National Institute of Public Health in Prague (WHO collaborating centre). Blood samples were examined using ELISA, Western blot and PCR.

Myocardial bioptic samples were fixated in 4% paraformaldehyde with 1.5% glutaraldehyde in 0.1 M buffer cacodylate solution for 5 hours in ambient temperature, washed three times in cacodylate buffer solution and then fixated in 1% osmic acid in 0.1 M buffer cacodylate solution for 1 hour. Then the fixated pieces of tissue underwent dehydration using ascending alcohol series during time intervals 3 times 5 minutes from 60% up to absolute alcohol, then saturated in the mixture of alcohol – Epon 812 in ratio 3:1, 1:1, 1:3, and then were polymerized in

Table 1 – 17 patients (13 men, 4 women), average age 58 years (range 43–76 y.)

Order	Sex M/W	Age	Dg.	ELISA IgM	ELISA IgG	WB	PCR	ELISA IgM	ELISA IgG	WB	PCR	Tick/ EM	Biopsy
1.	M	74	DCMP	156	129	neg.	+	*	*	*	*	+/0	+
2.	M	58	DCMP	333	319	neg.	neg.	478	428	bound	+	no	+
3.	M	68	DCMP	173	253	neg.	+	202	498	neg.	+	no	+
4.	M	49	DCMP	125	201	neg.	+	*	*	*	*	no	0
5.	W	43	DCMP	490	533	bound.	neg.	404	983	bound.	neg.	+/0	+
6.	M	56	DCMP	225	454	bound.	+	240	983	neg.	neg.	+	+
7.	M	44	DCMP	233	100	neg.	+	147	451	neg.	neg.	no	0
8.	W	74	DCMP	271	800	bound.	+	254	842	neg.	+	no	0
9.	M	43	DCMP	508	470	bound.	+	506	713	bound.	neg.	no	+
10.	M	61	DCMP	342	977	neg.	+	*	*	*	*	no	0
11.	M	64	DCMP	184	390	+	+	*	*	*	*	no	0
12.	M	56	DCMP	205	558	neg.	neg.	151	360	bound.	+	no	+
13.	W	64	DCMP	155	468	neg.	+	*	*	*	*	no	+
14.	M	76	DCMP	78	754	bound.	*	*	*	*	*	+/0	+
15.	M	54	DCMP	121	654	neg.	+	196	631	neg.	neg.	no	0
16.	M	51	DCMP	359	669	neg.	+	*	*	*	*	+/0	0
17.	W	59	DCMP	147	169	neg.	bound.	180	308	bound.	neg.	no	+

* = no control test, ELISA IgM 900 = negative, 900–1.000 = boundary value, > 1.000 = positive, ELISA IgG 700 = negative, 700–900 = boundary value, > 900 = positive, WB bound. = boundary value of the Western blot, Bio + = electronmicroscopy detection of Borrelia in the myocardial biopsy, Bio 0 = without detection of Borrelia in the myocardial biopsy, Tick (EM) = tick bite/eruption of the erythema migrans

pure resin Epon 812 at temperature 60–70 ^{0}C for 4 days. Semi-thick and ultrathin slices were sliced by ultramicrotome LKB 812 and examined by a light microscope Leitz and by an electron microscope Jeol 200CX. Ultrathin slices were stained by uranyl acetate and lead citrate.

Results

In total, 33 patients were examined: 10 females, 23 males with mean age 57.5 years (range 24–76 years). 16 patients were excluded from the study owing to the absence of signs of Bb. 17 patients were enrolled in the study – 4 females, 13 males with mean age 58 years (range 43–76 years), in whom the presence of Bb was proved using detection of DNA of pathogenic Borrelias or using electronmicroscopic detection of Spirochaetae in myocardial bioptic samples (Table 1).

In some samples, not only Borreliae, but also their special forms called cysts were detected (Figure 1).

Electronmicroscopic detection of Spirochetae was successful in 10 patients, in 8 out of them DNA of pathogenic Borreliae was proved as well (Figure 2).

Only presence of DNA of pathogenic Borrelias was confirmed in 7 out of 17 patients.

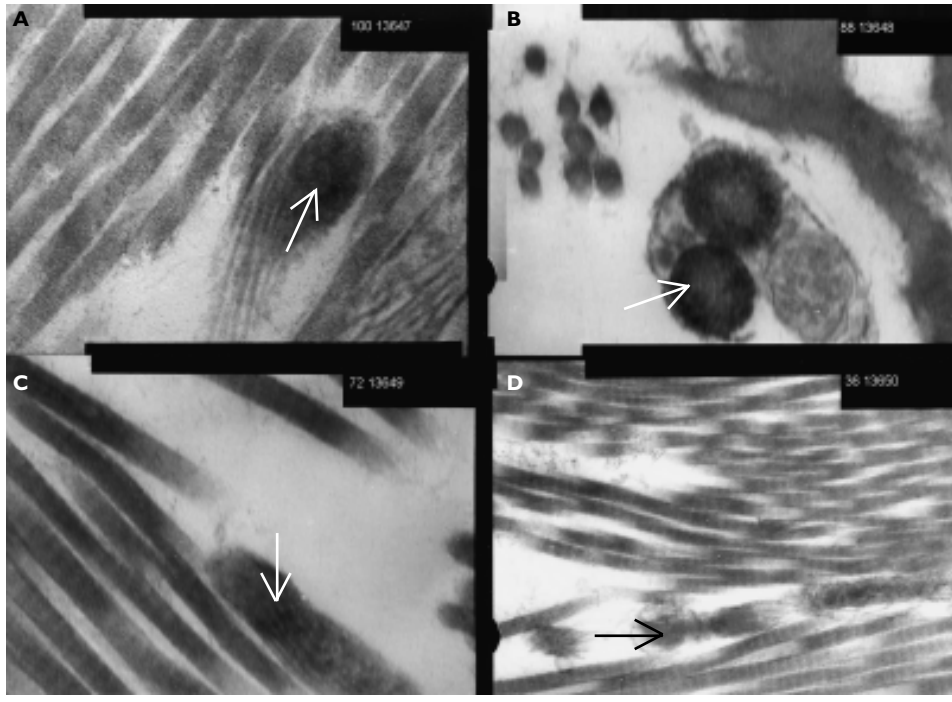

Figure 1 – A: a cyst between collagen fibres, oblique cut (magnified 100 000x); B: cysts (magnified 88.000x); C: Borrelia between collagen fibres (magnified 88 000x); D: Borrelia between collagen fibres (magnified 36 000x).

Discussion

This pilot study is based on experience with previous patients examined in the past years. During years 1994–2005, 3 patients with DCMP were followed-up and examined, the participation of Bb in the development of the late form of LC was confirmed in all of these patients. The patients' age was 45, 54 and 58 years at the moment of enrolment in the study. Two persons had cutaneous form of LB, so called erythema migrans (EM), and were treated with antibiotics in the early phase. The introductory examination included complete cardiological investigation (ECG, Holter ECG monitoring, echocardiogram, myocardial perfusion scan, selective coronarography), and complete laboratory screening including antibodies to Borrelia burgdorferi examined by ELISA, Western blot and PCR in the National Reference Laboratory for LB. Clinical findings and laboratory tests including direct detection of Bb in myocardium (in one female patient), and positivity of Bb by ELISA method, Western blot, PCR (in two patients), confirmed unequivocal participation of Bb infection in development of DCMP, which necessitated orthotopic heart transplantation in one patient. Ischemic heart disease and other possible conditions concordant with exclusion criteria of this pilot study were ruled out. The mentioned experience was formerly reported by our authors [14, 15].

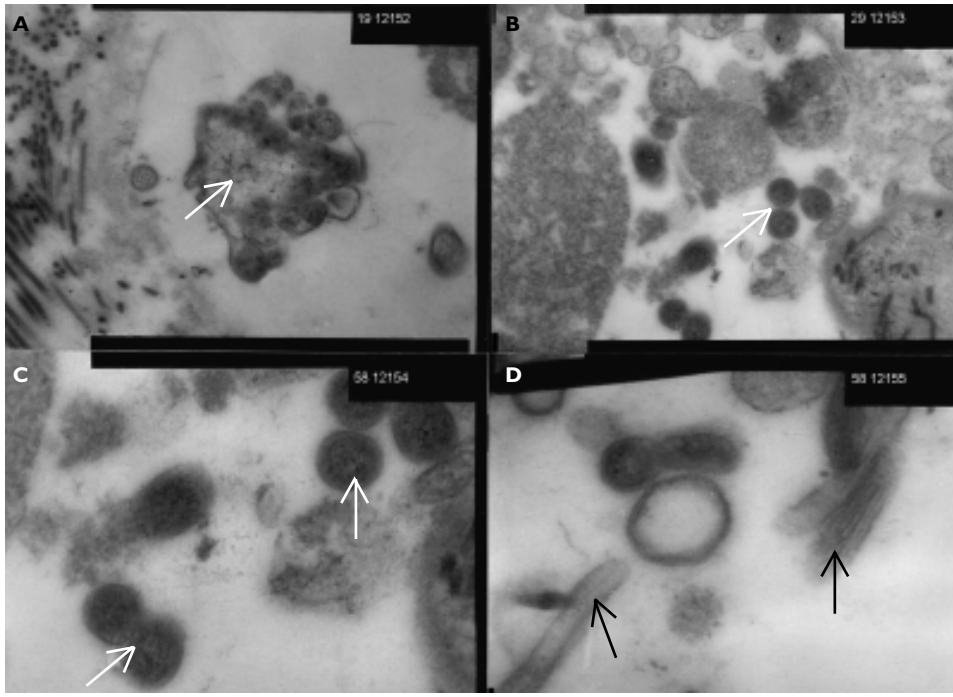

Figure 2 – A: a cyst with the remainder of Borrelia (magnified 19.000x); B: Anaplasma phagocytophillum / Ehrlichia/ (magnified 29.000x); C: Borrelia + Ehrlichia, cross cut (magnified 58.000x); D: Borrelia (magnified 58.000x).

Our persuasion about participation of BI in myocarditis with possible consequences for cardiac conduction system was strengthened by other reports from Austria, where the authors additionally examined 55 patients with coronary heart disease for antibodies to Bb, trying to elucidate the coincidence of probands' seropositivity in the regions endemic for LB. Four patients were positive. The marked difference in seropositivity between the patients with dilated cardiomyopathy and coronary heart disease (29.6% : 7.3%) provided other evidence not only about relation of LB to DCMP. Borrelia infection as a possible etiologic agent of DCMP is discussed by other authors [16, 17]. Remarkable but still isolated report was published by Glasser and co-workers. Two cases of coronary artery aneurysm are supposed to be associated with Lyme borreliosis as etiological agent, possibly as the late consequence [18].

Even if several thousands of reports deal with LB, and the results of skin biopsies with cultivation or PCR (50–70%) are rather well known, along with the results from synovial fluid or cerebrospinal liquor (50–70% with PCR), however, as regards myocardium, no larger study based on confirmation of DNA of pathogenic Borrelias is available, with exception of the already cited articles by Austrian authors. Likewise, there is lack of reports focused on Spirocheate detection in Germany [19, 20, 21]. It is conceivable, because myocardial biopsy is indicated solely in such cases, in which etiopathogenesis of cardiomypathy is not apparent, or if it emerges as probable during routinely performed coronarography.

The sample size of the LB-DCMP study is small and fails to warrant conclusions being able to fulfil premises within the study design. We are aware of the fact that myocarditis leading to development of DCMP may be caused by different etiology in which numerous bacteria, Rickettsiae or other agents may be involved. Economic expensiveness of the screening of the afore mentioned agents exceeded the possibilities of this study, which was designed as a pilot one. Even if the sample seems relatively small, evaluated by absolute numbers, considered low incidence of DCMP is outweighed by the importance of the obtained results. We suppose that the study brings useful information that may launch a larger, preferably multicentric study, being able to evidence, in addition to Bb detection, the influence of other infectious agents necessary for confirmation of the role that Bb plays in etiopathogenesis of DCMP. The urgency of the problem is underscored by the fact that approximately 70% of patients with DCMP die within 5 years.

Conclusion

The findings of our pilot study confirmed very probable participation of Borrelia infection in the development of dilated cardiomyopathy.

Thus, the obtained results may be a platform for multicentric study that will be launched in 2008 with participation of 4 university clinics of cardiology.

Footnotes

The first detection of Borrelia cyst was reported in 1992 (A. G. Barbour, [22]). It arises by coiling of Borrelia and by its gradual resorption. The envelope is created by flagella and a superficial wall. Its "transformation" back to Borrelia is triggered by various conditions (viral disease, corticoid therapy, immunopathy).

Anaplasma phagocytophillum = formerly Ehrlichia phagocytophillum, a causal organism of ehrlichiosis, a tick-borne zoonosis (Ixodes genus). It is an obligatorily intracellular bacteria (it infects granulocytes), it was reported in 1994 for the first time in patients from Minnesota and Wisconsin. Because of the identical vector, concomitant positive detection of Borrelias and Ehrlichias has reported. Both infections show similar clinical manifestations (fever, headache, fatigue, later myalgia, arthralgia and maculopapulous exanthema), and laboratory findings (thrombocytopenia, leukopenia, anaemia and increase in serum transaminases), [23].

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