Tick – Borne Infections as a Cause of Heart Transplantation

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A b s t r a c t

Many bacterial species can be a cause of various heart diseases, such as: *Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, *Bartonella* spp., *C. burnetii* and *Rickettsia* spp. The aim of the present studies was to establish if any tick-borne infections can contribute to serious heart disorders resulting in the need for heart transplantation. Myocardium, aortic and mitral valve samples from hearts removed from patients undergoing heart transplantation were tested. The presence of *Bartonella* spp., *Borrelia afzelii* and *C. burnetii* bacteria in malfunctioning human hearts has been shown. DNA of *Bartonella* spp., *B. burgdorferi* and *C. burnetii* were detected in various parts of tested hearts. DNA of *B. afzelii* and *Bartonella* spp. were found in the aortic valves. DNA of *C. burnetii* was detected in the myocardium. Mixed infections with *Bartonella* spp. and *C. burnetii* were also observed. Obtained results indicate that diagnosis of *Bartonella* spp., *B. burgdorferi* *C. burnetii* and *Rickettsia* spp. infections should be considered in cases of infectious endocarditis with negative blood cultures.

K e y w o r d s: infective endocarditis, bartonellosis, Q fever, Lyme boreliosis

Many bacterial species and viruses can be a cause of various heart diseases. The pathogens that trigger these disorders are very often fastidious, uncultured bacteria, such as: *Borrelia burgdorferi* sensu lato, *Coxiella burnetii*, *Bartonella* spp. and *C. burnetii*. Myocardium, aortic and mitral valve samples from hearts removed from patients undergoing heart transplantation were tested. The presence of *Bartonella* spp., *Borrelia afzelii* and *C. burnetii* bacteria in malfunctioning human hearts has been shown. DNA of *Bartonella* spp., *B. burgdorferi* and *C. burnetii* were detected in various parts of tested hearts. DNA of *B. afzelii* and *Bartonella* spp. were found in the aortic valves. DNA of *C. burnetii* was detected in the myocardium. Mixed infections with *Bartonella* spp. and *C. burnetii* were also observed. Obtained results indicate that diagnosis of *Bartonella* spp., *B. burgdorferi* *C. burnetii* and *Rickettsia* spp. infections should be considered in every case of infectious endocarditis with negative blood cultures.

In this study 17 fixed in formalin samples of myocardium, 23 of aortic and 22 mitral valve from twenty four hearts removed from patients undergoing heart transplantation, and one frozen endomyocardial biopsy sample were tested. They were collected from 2006 to 2010. Each tissue sample was homogenized. DNA was extracted with the QIAamp Tissue kit (QIAGEN Gmbh, Hilden, Germany) according to the manufacturer’s descriptions.

*B. burgdorferi* DNA was detected with L2 and P1 primers for the 16S rRNA and with OA149 and OA319 specific primers for the OspA gene fragments characteristic for all *Borrelia* species: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* (Million et al., 2010; Podsidiy et al., 2003). The primers BhCS.781p and BhCS.1258n were used to amplify a 400-bp fragment of the *Bartonella* spp. citrate synthase gene (Nilsson et al., 2005). Detection of *Rickettsia* spp. DNA was performed using primers RpsCS.409d and RpsCS.1258n for conserved regions of the citrate synthase gene (Nocton et al., 1994). *C. burnetii* DNA was detected with primers...
HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.1% gelatin, 200 μM dNTPs, 50 pmol of each primer and 1.5 U Taq DNA polymerase (Perkin-Elmer Cetus, USA). An aliquot of 5 μl of extracted DNA template was added to each reaction mixture. Each PCR test included negative (water) and positive controls containing DNA of B. afzelii, B. garini, B. henselae, C. burnetii Henzerling strain, Rickettsia conori H24 strain, from the collection of the NIH. For B. burgdorferi and Rickettsia spp. the cycling conditions were as follows: 3 min at 95°C, followed by 40 cycles of 1 min denaturation at 95°C, annealing 1 min at 55°C, elongation 1 min at 72°C and final elongation 7 min at 95°C. For Bartonella spp. the cycling conditions were as follows: 10 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 57°C, 2 min at 72°C and finally 10 min at 72°C. For C. burnetii the cycling conditions were as follows: 3 min at 95°C, followed by 40 cycles of 1 min at 95°C, 1 min at 57°C, 1 min at 72°C and finally 7 min at 95°C. PCRs were performed in a thermal Mastercycler ep (EP Gradient, Eppendorf AG, Hamburg, Germany). Isolated DNA from strains collected in the National Institute of Public Health – National Institute of Hygiene including: Salmonella thadar, Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Yersinia enterocolitica, Leptospira biflexa and Treponema pallidum (kindly provided from Institute of Venerology, Medical University of Warsaw) were used as a negative controls. All amplicons were analyzed in electrophoresis in 1.5% agarose gel stained by 4% ethidium bromide. 

amplicons with the ABI 377 DNA Analyzer (Applied Biosystem, USA) according to the manufacturer’s recommendations. All sequences were edited using Autoassembler software (Applied Biosystem, USA) and identified using the BLAST software by comparison with sequences available in GenBank.

DNA of Bartonella spp., B. burgdorferi and C. burnetii was detected in various parts of the hearts of patients undergoing heart transplantation. The study group consisted of 4 patients suffered from severe dilated cardiomyopathy of different etiology. DNA of Borrelia afzelii was found in the aortic valves of two patients (B. afzelii 16S rRNA gene, B. afzelii OspA gene). Mixed infections were found in two other patients. In the first, DNA characteristic for Bartonella spp. was detected in the aortic valves and DNA of C. burnetii in the myocardium. In the second patient, DNA characteristic for Bartonella spp. was detected in the mitral valve and DNA of C. burnetii was detected in the myocardium and the aortic valves (Table II). DNA of Rickettsia spp. was not found in any tested material.

Two sequences of is1111 gene partially sequenced over a total 74 nucleotide positions showed 98% (myocardium sample) and 100% (myocardium and aortic valve samples from the same patient) nucleotide identity between detected strains and C. burnetii strains: Nov1105 (Accession number EF541175.1), Nov11506 (EF541174.1). Mng 3602 (DQ469888.1). One sequence of OspA gene partially sequenced over a total 427 nucleotide positions showed 98% (aortic valve) nucleotide identity between detected strains and B. afzelii strains: Nov1105 (Accession number EF541175.1), Nov11506 (EF541174.1). Mng 3602 (DQ469888.1). One sequence of OspA gene partially sequenced over a total 147 nucleotide positions showed 99% (aortic valve) nucleotide identity between detected strains and B. afzelii strains: ACA-1 plasmid Ip54 (Accession number CP001247.1), PKo plasmid Ip60 (CP000396.1). One sequence of citrate synthase (gltA)
gene partially sequenced over a total 235 nucleotide positions showed 99% (aortic valve) nucleotide identity between the detected strains and Bartonella sp. strains: clone G10 citrate synthase (gltA) gene (Accession number HM116785.1), BCF02 citrate synthase (gltA) gene (GU056189.1), Sr3sk (AY587980.1). One sequence of citrate synthase (gltA) gene partially sequenced over a total 233 nucleotide positions showed 99% (mitral valve) nucleotide identity between detected strains and Bartonella sp. strains: clone G10 (Accession number HM116785.1).

Our research has shown the presence of Bartonella spp., B. afzeli and C. burnetii bacteria in malfunctioning human hearts. The detected pathogens, occurring in ticks in the natural environment, have a clinical importance in cardiology (Brouqui and Raoult, 2001; Nilsson et al., 2005). It is well known that C. burnetii, an etiologic agent of Q fever, is responsible for difficult to cure endocarditis, with high mortality rate (Chmielewski et al., 2003). Generally, C. burnetii infections are located on heart valves. In the presented study the bacteria have also been found in the myocardium. This confirms the rarely cases described when the pathogen is responsible for myocarditis. In the last decade of the twentieth century heart failures due to B. burgdorferi and Bartonella spp. were described. Dilated cardiomyopathy (DCM), conduction and rhythm disturbances have been observed in Lyme endocarditis patients, whereas endocarditis and myocarditis in bartonellosis patients (Brouqui and Raoult, 2001; Podsiadly et al., 2007). The obtained results indicate that among patients with cardiac diseases, infections caused by B. burgdorferi, C. burnetii and Bartonella spp., should be obligatorily tested for. Our results indicate that father studies are needed. It is necessary to exam if the detected bacteria are the direct cause of cardiologic complications or they accompany the other decisive factors.

Acknowledgments

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<table>
<thead>
<tr>
<th>No. patients</th>
<th>Clinical sample</th>
<th>Borrelia afzeli</th>
<th>Bartonella spp.</th>
<th>Coxiella burnetii</th>
<th>Clinical symptoms</th>
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<tr>
<td>1</td>
<td>Aortic valve</td>
<td>positive*</td>
<td>negative**</td>
<td>negative</td>
<td>coronary artery disease</td>
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<tr>
<td>2</td>
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<td>negative</td>
<td>negative</td>
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<tr>
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<td>positive</td>
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<td>Mitral valve</td>
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<tr>
<td>4</td>
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<td>negative</td>
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<td>myocarditis</td>
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<tr>
<td></td>
<td>Aortic valve</td>
<td>negative</td>
<td>positive</td>
<td>negative</td>
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* Bacterial DNA detected with polymerase chain reaction (PCR)

** Bacterial DNA not detected

Rickettsia spp. DNA was not detected

Table II

Location of Borrelia afzeli, Bartonella spp. and Coxiella burnetii DNA in removed hearts of transplant recipients suffering from dilated cardiomyopathy

<table>
<thead>
<tr>
<th>No. patients</th>
<th>Clinical sample</th>
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