SHORT COMMUNICATION

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. The pathogens that trigger these disorders are very often fastidious, uncultured bacteria, such as: Borrelia burgdorferi sensu lato, Coxiella burnetii and Bartonella spp. 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 Rickettsia spp. (Brouqui and Raoult, 2001). This is the reason why infections with these bacteria are very often undiagnosed. For example it is estimated that over 70% of infective endocarditis cases with negative blood cultures are related to C. burnetii infections (Chmielewski et al., 2003). The following symptoms: myocarditis, endocarditis, pancarditis, perimyocarditis, dilated cardiomyopathy (DCM), conduction and rhythm disturbances, atherosclerotic, cardiovascular and valvular disease may indicate the bacterial etiology of the disease and detection of significant titers of specific antibodies allows identifying the origin of the disease (Brouqui and Raoult, 2001; Fournier and Raoult, 2003; Million et al., 2010). 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. Moreover, it should be established whether 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; Podsadly et al., 2003). The primers BhCS.781p and BhCS.1137n 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 RpCS.409d and RpCS.1258n for conserved regions of the citrate synthase gene (Nocton et al., 1994). C. burnetii DNA was detected with primers

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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, 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 B. burgdorferi 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 of 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: CbuK_Q154 (Accession number CP001020.1), CbuG_Q212 (CP000109.1), RSA331 (CP000890.1). The sequence of 16S rRNA 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 lp54 (Accession number CP001247.1), PKo plasmid lp60 (CP000396.1). One sequence of citrate synthase (gltA)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primers</th>
<th>Fragment gene (size, bp)</th>
<th>Nucleotide sequences</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borrelia burgdorferi</td>
<td>L2</td>
<td>16S rRNA (600)</td>
<td>5'-GGTCAGAGACTGACGGCTAGT 5'-TCCGTTTGTGACGGCATTG</td>
<td>Chmielewski et al. 2003</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td></td>
<td>5'-TTATGAAAAATATTTATGGGAAT 5'-CTTTAAGCTCAAGCTTGTCTACGT</td>
<td>Noctor et al. 1994</td>
</tr>
<tr>
<td></td>
<td>OA149</td>
<td>OspA gene</td>
<td>5'-GGGGACCAGCTCATGGGTG 5'-AATGCAAAAAAGAACAGTAAACA</td>
<td>Podsiadly et al. 2007</td>
</tr>
<tr>
<td>Bartonella spp.</td>
<td>BhCS.781p</td>
<td>citrate synthase gene</td>
<td>5'-CGCTCTGGTTATGGCAGC 5'-CCAACAACACCTCTTATTC</td>
<td>Fournier et al. 2003</td>
</tr>
<tr>
<td></td>
<td>BhCS.1137n</td>
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<tr>
<td>Coxliella burnetii</td>
<td>isIIIIf</td>
<td>htpAB</td>
<td></td>
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<td></td>
<td>isIIIr</td>
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gene partially sequenced over a total 235 nucleotide positions showed 99% (aortic valve) nucleotide identity between the detected strains and \textit{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 \textit{Bartonella} sp. strains: clone G10 (Accession number HM116785.1).

Our research has shown the presence of \textit{Bartonella} spp., \textit{B. afzelli} and \textit{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 \textit{C. burnetii}, an etiologic agent of Q fever, is responsible for difficult to cure endocarditis, with high mortality rate (Chmielewski et al., 2003). Generally, \textit{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 \textit{B. burgdorferi} and \textit{Bartonella} spp. were described. Dilated cardiomyopathy (DCM), conduction and rhythm disturbances have been observed in Lyme borreliosis 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 \textit{B. burgdorferi}, \textit{C. burnetii} and \textit{Bartonella} spp., should be obligatorily tested for. Our results indicate that father studies are needed. It is necessary to examine if the detected bacteria are the direct cause of cardiology complications or they accompany the other decisive factors.

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\textbf{Literature}


