Cardiac Valves in Patients with Q Fever Endocarditis: Microbiological, Molecular, and Histologic Studies

Hubert Lepidi,1,2 Pierre Houpikian,1 Zhongxing Liang,1 and Didier Raoult1

1Unité des Rickettsies, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6020, Institut Fédératif de Recherche 48, and 2Laboratoire d’Histologie, Faculté de Médecine, Université de la Méditerranée, Marseille, France

The pathologic features of Q fever endocarditis, which is caused by Coxiella burnetii, were histologically evaluated in cardiac valves from 28 patients. We used quantitative image analysis to compare valvular fibrosis, calcifications, vegetations, inflammation, and vascularization due to Q fever endocarditis with that due to non–Q fever endocarditis and valvular degeneration. We also studied the presence of C. burnetii in valves by immunohistochemical analysis, culture, and polymerase chain reaction (PCR). Histologically, Q fever endocarditis was characterized by significant fibrosis and calcifications, slight inflammation and vascularization, and small or absent vegetations. Despite antibiotic treatment, non–statistically significant variations at the histologic level were observed. These pathologic features could be confused with noninfectious valvular degenerative damage. We found that the detection of C. burnetii in cardiac valves by immunohistochemical analysis, culture, and PCR decreased significantly only after 1 year of antibiotic treatment, which emphasizes the long persistence of this organism in valve tissues. Pathologic and immunohistochemical analyses may contribute to the diagnosis of Q fever endocarditis.

Q fever is a worldwide zoonosis caused by Coxiella burnetii, a strictly intracellular bacterium that lives in the monocyte/macrophage, its host phagocytic cell. The main characteristic of Q fever is its clinical polymorphism [1]. Q fever is commonly divided into an acute and a chronic form. Acute Q fever corresponds to primary infection with C. burnetii. One-half of patients with acute Q fever are asymptomatic. The most common syndromes observed in acute Q fever are prolonged fever of unexplained origin, granulomatous hepatitis, and atypical pneumonia [2]. Infective endocarditis is the major manifestation of chronic Q fever [2].

C. burnetii was recognized as one of the bacteria that cause blood culture–negative endocarditis [3]. Q fever endocarditis accounts for at least 5% of all cases of endocarditis diagnosed in France [4]. Currently, most cases of Q fever endocarditis are diagnosed serologically, by detection of specific antibodies [5]. High levels of anti–phase I antibodies are found in chronic Q fever, whereas anti–phase II antibodies predominate during acute Q fever [6]. Diagnosis of Q fever endocarditis can also be achieved by C. burnetii isolation in cell culture, polymerase chain reaction (PCR), or immunohistochemical examination [7–9].

Because symptoms of Q fever endocarditis are protean and not specific, diagnosis is often delayed, resulting in an increasing mortality rate. Moreover, because the pathologic characteristics of Q fever endocarditis rarely have been reported in the literature [7, 10–14], it is often difficult to recognize the disease on the basis of pathologic evidence. We examined data on the clinical and pathologic features of 28 cases of Q fever endocarditis selected from the information col-
lected by our reference center for rickettsial diseases (Unité des Rickettsies, Marseille, France). This sample was large enough to allow investigation of pathologic characteristics associated with Q fever endocarditis. In the present article, we used quantitative image analysis to describe these pathologic characteristics by analyzing several histologic parameters, inflammation, and neovascularization in cardiac valve tissues. Moreover, we performed immunohistochemical testing for localization of *C. burnetii* in resected cardiac valves with a monoclonal antibody (MAb), using a peroxidase-based method and paraffin-embedded tissues. We compared bacterial detection in valve tissues by immunohistochemical examination, culture, and PCR in patients with different durations of antibiotic treatment.

**PATIENTS, MATERIALS, AND METHODS**

**Case definition and patients.** Patients were considered to have definite blood culture–negative endocarditis if the results of standard blood cultures were negative and if clinical and echocardiographic findings met the modified Duke Endocarditis Service criteria for the diagnosis of Q fever endocarditis [4]. Antibody titers in patient serum samples were determined by immunofluorescence assay [5]. Patients who exhibited an IgG titer $\geq 800$ against *C. burnetii* phase 1 were considered to have chronic Q fever. Such a titer has been demonstrated to have a positive predictive value of 98% and a sensitivity of 100% and is considered to be the major criterion for diagnosis of Q fever endocarditis [4, 5]. At least 2 serum samples were tested before patients were included in the study. For each patient in whom Q fever endocarditis was suspected, 28 samples were collected on Vero cell monolayers, purified, and inactivated in 0.1% formalin, as described elsewhere [13, 18]. The procedure for the production of MAbs has been described elsewhere [19, 20]. In brief, 6-week-old BALB/c mice were inoculated intraperitoneally with inactivated *C. burnetii* suspended in PBS. The supernatants of the hybridomas were screened for antibodies to *C. burnetii* by immunofluorescence, using the 20 *C. burnetii* strains as antigens. Three representative hybridomas, including Ch10B10, were subcloned by limiting dilution. Serum samples from immunized and nonimmunized mice were used as positive and negative controls, respectively. Four *Bartonella* strains (2 *B. henselae, 1 B. quintana, and 1 B. elizabethae strain) and a *Tropheryma whippelii* strain were also used as controls to test the specificity of MAbs by immunofluorescence (data not shown). SDS-PAGE and Western blotting were performed according to a modification of the method described by Liang et al. [21] and Laemmli [22].

**Immunohistochemical analysis.** Immunohistochemical analysis was performed on paraffin-embedded valve sections with the anti-*C. burnetii* Ch10B10 mouse MAb. The immunohistochemical procedure, in which an immunoperoxidase kit was used, has been described elsewhere [16]. For each tissue section, a negative control was performed using an irrelevant mouse MAb. To evaluate the macrophage burden in valve tissue and neovascularization, paraffin sections were stained with the macrophage marker CD68 (Dako) at a working dilution of 1:1000 and with a polyclonal antibody to the vascular deter-
Cardiac Valves in Q Fever Endocarditis

Table 1. Pathologic features of infectious endocarditis in native, bioprosthetic, and mechanical cardiac valves.

<table>
<thead>
<tr>
<th>Disease, valve type, patient group</th>
<th>No. of patients</th>
<th>Extensive fibrosis with or without calcifications</th>
<th>Large vegetations</th>
<th>Extensive inflammation and vascularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non–Q fever endocarditis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>25</td>
<td>3 (12)</td>
<td>20 (80)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Bioprosthetic</td>
<td>10</td>
<td>6 (60)</td>
<td>7 (70)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Q fever endocarditis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>16</td>
<td>9 (66.2)</td>
<td>3 (18.7)</td>
<td>3 (18.7)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>2 (40)</td>
<td>2 (40)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>2 and 3</td>
<td>11</td>
<td>7 (63.6)</td>
<td>1 (9)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Bioprosthetic</td>
<td>10</td>
<td>7 (70)</td>
<td>2 (20)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2 (66.6)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>2 and 3</td>
<td>7</td>
<td>5 (71.4)</td>
<td>1 (14.2)</td>
<td>1 (14.2)</td>
</tr>
<tr>
<td>Mechanical</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2 and 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Fibrosis and vascularization are not observed in bioprosthetic valves.
endocarditis, including 2 (40%) from group 1 and 1 (9%) from groups 2 and 3, showed the vegetations, large inflammatory infiltrates, and significant tissue destruction typical of infective endocarditis.

We quantitatively analyzed native valves from patients with Q fever endocarditis. The native C. burnetii–infected valves from group 1 were significantly more fibrotic ($P = .001$) and calcified ($P = .02$), but valvular vegetations and neovascularization were less extensive ($P = .001$ and $P = .003$, respectively) than in the control endocarditis group (figure 3). CD68 expression, which quantified macrophage surface area, was dramatically lower in valve tissue from patients with Q fever endocarditis than in samples from the control endocarditis group ($P = .01$; figures 3 and 4). The native valves from patients with Q fever endocarditis in group 1 were also quantitatively compared with those from groups 2 and 3. Morphologic changes

---

**Figure 1.** Aortic valve from a patient with Q fever endocarditis. Note the extensive fibrosis of the connective valve tissue (*) and focal calcifications on the valve surface (arrow). (Hematoxylin-eosin-saffron stain; original magnification, $\times 100$.)

**Figure 2.** Mitral valve from a patient with Q fever endocarditis. Note the small vegetation on the surface of the valve tissue (*) with a mononuclear inflammatory cell infiltrate (arrow). (Hematoxylin-eosin-saffron stain; original magnification, $\times 250$.)
in groups 2 and 3 were minimal, despite antibiotic treatment, and were not statistically significant. After treatment, heart valves showed a slight increase in fibrosis and calcification and a tendency to have smaller vegetations, inflammation, and vascularization (figure 3). The morphologic features seen in native valves from patients with Q fever endocarditis were very similar to those of degenerative native valves (figures 3 and 5), except that degenerative valves lacked vegetations.

Seven (70%) of the 10 C. burnetii–infected bioprosthetic valve specimens showed large calcifications on histologic examination (table 1). In 6 valve specimens (60%), vegetations were small in size, and minimal mononuclear cell inflammation was observed. In 2 specimens (20%) from groups 2 and 3 that were devoid of vegetations, an infective process was not histologically apparent. Only 2 bioprosthetic valves (20%) from patients with Q fever endocarditis, 1 (33.3%) of the 3 from group 1 and 1 (14.2%) of the 7 from groups 2 and 3, showed typical histologic features of valvular infection (vegetations, large inflammatory infiltrates, and significant tissue destruction). The 2 mechanical valves from patients with Q fever endocarditis were characterized by a lack of vegetations and calcifications, small mononuclear cell inflammatory infiltrates, and discrete neovascularization. Quantitative analysis of the 3 bioprosthetic C. burnetii–infected valves from group 1 showed that they were significantly less inflamed ($P = .01$), as shown by CD68 expression, than bioprosthetic valves from the control endocarditis group and had smaller vegetations ($P = .01$; figure 6). In contrast, the quantities of calcifications were similar in valves from the Q fever and control endocarditis groups (figure 6). We could not quantify fibrosis and neovascularization in bioprosthetic valves, because they do not contain living tissue. Morphologic changes in the bioprosthetic valves from patients with Q fever endocarditis were similar after treatment, and we noted a tendency toward reduction in the sizes of vegetations and inflammation. On the other hand, degenerative bioprosthetic valves were significantly more calcified ($P = .01$) than were bioprosthetic valves from patients with Q fever endocarditis but were as inflamed and lacked vegetations (figure 6).

The large calcifications seen in degenerative bioprostheses were not surprising, because this damage, along with the degenerative process of the connective tissues, is the major complication associated with this type of prosthesis [24]. Periodic acid–Schiff, Brown–Hopps/Brown–Brenn Gram, Grocott–Gomori methenamine silver, and Warthin–Starry staining showed no microorganisms in valve tissues from patients with Q fever endocarditis or in degenerative valves.

**Detection of C. burnetii by cell culture, PCR, and immunohistochemical analysis.** C. burnetii was isolated by culture from 18 valve specimens (64.2%) and identified by PCR in 21 specimens (75%) from patients with Q fever endocarditis (table 2). C. burnetii was detected in $>80\%$ of the valves that were excised before the beginning of antibiotic therapy and during

---

**Figure 3.** Quantification of histopathologic changes in native valves from patients with non–Q fever endocarditis ($n = 25$), Q fever endocarditis (groups 1 [$n = 5$] and 2 and 3 [$n = 11$]), and degenerative valvular disease ($n = 25$). Fibrosis, calcification, and vegetations were quantified in hematoxylin-eosin-saffron–stained valve tissue sections, and CD68 and factor VIII staining were quantified after immunostaining. Quantification of each parameter was evaluated by computer-assisted analysis of digitized microscopic images. Results were normalized and expressed as a percentage of total valve surface area. Columns represent mean values ± SE.
Figure 4. Focal and small inflammatory infiltrates with immunostained macrophages (arrows), representing a small area in the whole valve tissue surface. The valve stroma is reorganized and fibrotic (*). [Immunoperoxidase staining with an anti-CD68 monoclonal antibody; original magnification, ×100.]

Figure 5. Section of an aortic native valve with degenerative damage. Note the fibrosis of connective tissue (*), the large inflammatory infiltrates (arrow) composed mainly of mononuclear cells, and neovascularization. [Hematoxylin-eosin-saffron stain; original magnification, ×100.]

The first year after therapy. In contrast, after 1 year of antibiotic treatment, the percentages of positive detection with culture and PCR decreased dramatically, to only 22.2% and 33.3%, respectively (P = .01 and P = .03, respectively; table 2).

The MAb Cb10B10, identified by Western immunoblot as an IgG1 that reacts with a 25–26-kDa band (data not shown), was selected for immunohistochemical studies of cardiac valve tissues. Of the 28 valve specimens from patients with Q fever endocarditis, C. burnetii was identified in 9 (32.1%) (table 2). None of the controls showed immunoreactivity, which confirms the specificity of the antibody used. In Q fever endocarditis, bacteria were seen as coarse granular immunopositive material in the macrophage cytoplasm (figure 7). C. burnetii could only be visualized within regions of inflammation, as small, focal
collections of infected mononuclear cells. The detection of *C. burnetii* by immunohistochemical examination correlated negatively with the duration of the antibiotic therapy and markedly decreased after 1 year of treatment; however, these differences were not statistically significant, probably because of the small number of cases studied (table 2).

**DISCUSSION**

Fever, positive blood cultures, and the finding of valvular vegetations on echocardiographic examination are the usual indicators of infective endocarditis. The Duke criteria, including these 3 indicators, are widely used for the diagnosis of infective endocarditis [25]. However, the protean and nonspecific clinical manifestations, the sterility of routine blood cultures, and the frequency with which echocardiography failed to detect vegetations often delay the diagnosis for months to years after *C. burnetii* infection of cardiac valves [2, 26]. Because Q fever endocarditis generally is associated with a poor prognosis and is fatal if untreated, an early diagnosis is needed [7, 27]. The diagnosis of Q fever can be established serologically or by cell culture [5, 28]. However, the role of the pathologist can be decisive in the recognition of endocarditis, especially when the microbiologist fails to isolate the causative microorganism. Examination of the infected valve, which can reveal vegetations, valvular inflammation, organisms, or other changes consistent with infective endocarditis, remains the reference standard for diagnosis of infective endocarditis. Histologic findings are included in the Duke [25] or the von Reyn [29] criteria for diagnosis of infective endocarditis. When considering the possibility of culture-negative endocarditis, the pathologist should first consider fastidious and slow-growing microorganisms, such as *C. burnetii* or *Bartonella* species [7, 16, 26, 30].

Q fever endocarditis in our series occurred in patients with previous valvular defects, bioprosthetic valves, or mechanical valves, as has been reported elsewhere [6, 31]. Most pathologic descriptions of Q fever endocarditis are based on individual case reports or series with few patients [7, 10–14, 32]. In this study, the largest cohort of patients with Q fever endocarditis was investigated at the histopathologic level. We found small

![Figure 6](image-url)

**Figure 6.** Quantification of histopathologic changes in bioprosthetic valves from patients with non–Q fever endocarditis (*n* = 10), Q fever endocarditis (groups 1 [*n* = 3] and 2 and 3 [*n* = 7]), and degenerative valvular disease (*n* = 25). Calcification and vegetations were quantified in hematoxylin-eosin-saffron–stained valve tissue sections, and CD68 staining was quantified after immunostaining. Each parameter was evaluated by computer-assisted analysis of digitized microscopic images. Results were normalized and expressed as a percentage of total valve surface area. Columns represent mean values ± SE.

**Table 2.** Comparison of methods for detecting *Coxiella burnetii* in valve tissues from patients with Q fever endocarditis, according to the duration of therapy with doxycycline and hydroxychloroquine.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>Culture</th>
<th>PCR</th>
<th>Immunohistochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>8 (88.9)</td>
<td>9 (100)</td>
<td>5 (55.5)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>8 (80)</td>
<td>9 (90)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2 (22.2)</td>
<td>3 (33.3)</td>
<td>1 (11.1)</td>
</tr>
</tbody>
</table>

**NOTE.** Members of group 1 received no antibiotic treatment; members of group 2 received antibiotic treatment for <1 year; and members of group 3 received antibiotic treatment for ≥1 year. PCR, polymerase chain reaction.
vegetations, mononuclear cell inflammation, extensive valvular fibrosis, and frequent calcifications in patients with Q fever endocarditis. These histologic features indicate a slow infectious process, similar to that observed with other blood culture–negative types of endocarditis, such as bartonellosis and Whipple disease [7, 17, 33].

Endocarditis is usually identified histologically by demonstration of vegetations and inflammatory reactions in valve tissue [17]. However, these major histologic features of endocarditis were minimal or absent in our series of patients with Q fever endocarditis, occupying, in several cases, only a small proportion of the valve tissue examined. Turck et al. [12] reported that small vegetations with insignificant valve damage were observed in 5 of 10 valves from patients with Q fever endocarditis. This suggests that the histologic features of Q fever endocarditis may be confused with degenerative damage, rather than being identified as an infectious process, as we showed them to be by quantitative analysis. When no vegetations are visible on echocardiographic examination (e.g., because vegetations are small or absent), the lack of clinical echocardiographic evidence of endocarditis can increase confusion. In 2 recent series, vegetations were found on echocardiography in only 13%–21% of patients with Q fever endocarditis [34, 35]. However, we showed that when vegetations are present histologically, they remain the main criterion for differentiating Q fever endocarditis from a valvular degenerative process, and they must be carefully sought by the pathologist examining the excised valve.

Serologic testing remains the reference technique for Q fever diagnosis [5]. Cultures for C. burnetii cannot be easily performed in most laboratories, and the technique is restricted to laboratories equipped for isolation of dangerous pathogens. PCR-based methods have also been successfully applied to detection of C. burnetii from tissue samples [36]. However, there is a risk in PCR of false-positive reactions caused by DNA contamination, and results still must be compared with histologic findings. On the other hand, C. burnetii is not observed in valve specimens when hematoxylin-eosin stain is used, and routine examination of resected valves is insufficient to establish a diagnosis of Q fever. Two studies describe the usefulness of the Gimenez and Macchiavello stains in detection of C. burnetii in cardiac valves [37, 38]. However, these special histologic stains are not specific.

Immunohistologic examination is able to demonstrate the presence of C. burnetii in specimens from patients with Q fever endocarditis [7, 39]. We generated MAbs to detect C. burnetii in tissue specimens. We used an MAb- and peroxidase-based immunodetection technique that better preserves morphology than do immunofluorescence techniques [40]. In agreement with other reports [7, 8, 31], the organisms were found only in an intracellular location, within the cytoplasm of macrophages of the inflammatory infiltrate. In spite of the small size of some lesions, the demonstrated persistence of C. burnetii is likely to elicit continued valvular inflammation that, in turn, causes progressive valvular damage. In our series, immunohistochemical examination was less sensitive than PCR and culture in demonstrating the organism in untreated patients. In patients in the early stages of Q fever
endocarditis, the number of bacteria may be too low to be detected by immunohistochemical examination. The fact that a high proportion of cardiac valves in our series showed minimal damage probably reflects early diagnosis of Q fever endocarditis by serologic testing. The mean time to diagnosis by experienced physicians has improved to 6 months, compared with 18 months in the older literature [27, 41]. The failure to detect *C. burnetti* in patients with Q fever endocarditis by immunohistochemical analysis may also result from sampling error. Resected valve tissues are frequently small, and the infective process may be confined to a small area. Because a portion of the sample is processed for microbiological testing, the material remaining for histologic examination may lack inflammatory areas with *C. burnetti*-infected macrophages. Thus, failure to find *C. burnetti* on immunohistochemical examination does not necessarily exclude the diagnosis of Q fever endocarditis. Regardless of the method used, detection of *C. burnetti* correlates with the duration of treatment and decreases significantly only after 1 year of appropriate antibiotic therapy. These results emphasize that *C. burnetti* can persist in valve tissue for >1 year after at least 18 months of treatment with doxycycline and hydroxychloroquine [23, 42]. Importantly, the inflammatory reaction associated with chronic Q fever endocarditis lacks well-formed granulomas. This observation suggests that patients with Q fever endocarditis could be unable to develop an effective cellular immune response against the bacterium, in contrast to that observed in the liver or bone marrow during acute Q fever [43–47].

In conclusion, we present the results of what is, to our knowledge, the largest series of Q fever endocarditis cases for which quantitative pathologic examination was performed and in which an immunohistochemical method for localization of *C. burnetti* on paraffin-embedded material was used. We showed that tissue damage with Q fever endocarditis is localized to only a small part of the valve. Because the lesions are small and discrete, they may be confused with degenerative changes. However, these lesions should be identified—they represent the early and treatable stages of chronic Q fever endocarditis.

**Acknowledgment**

We thank J. Stephen Dumler for reviewing the manuscript.

**References**

28. Von Reyn CF, Levy BS, Arbird RD, Friedland G, Crumpacker CS.


