Immunohistochemical detection of *Coxiella burnetii* in an aortic graft

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Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, a strictly intracellular and highly infectious bacterium that lives in the monocyte/macrophage, its host cell. Q fever is characterized by its clinical polymorphism, and develops as acute infections, some of which will evolve to chronic forms [1]. Patients with acute Q fever are more often asymptomatic. The most common syndromes observed in acute Q fever are prolonged fever of unexplained origin, granulomatous hepatitis, and atypical pneumonia. Although infective endocarditis is the main manifestation of chronic Q fever, the other chronic manifestations are infections of aneurysms or vascular prostheses, chronic infections after pregnancy, and other rare chronic forms such as osteomyelitis, chronic hepatitis, pseudotumour of the lung or infectious arthritis [2].

Because the symptoms of Q fever vasculitis are not specific, diagnosis is often delayed, resulting in an increased mortality rate [3]. Moreover, it is often difficult to recognize the disease on the basis of pathological evidence. We report in this study the first immunohistochemical detection of *C. burnetii* in an aortic graft using a peroxidase-based method and paraffin-embedded tissues.

**CASE REPORT, MATERIALS AND METHODS**

A 55-year-old man suffered from acute dissection of the thoracic aorta in June 2004. He received emergency surgery with implantation of an aortic Dacron endoprosthesis. Thereafter, he suffered from repeated thromboembolic complications such as kidney, spleen and cerebral infarction, and right popliteal artery embolus. Q fever was diagnosed serologically in September 2006, and he then received a second endoprosthesis replacing this infected previous endoprosthesis.

Q fever was diagnosed by serology. IgG, IgM and IgA titres to phases I and II of *C. burnetii* were estimated using an indirect microimmunofluorescence assay as previously described [4]. To prevent the presence of rheumatoid factors influencing the results, IgM antibodies were removed from the samples before titration of IgG and IgA. DNA was extracted from EDTA blood collected at the time of diagnosis using the QiaAmp tissue kit as recommended by the manufacturer (Qiagen, Hilden, Germany). Detection of *C. burnetii* was attempted by PCR amplification using primers targeting the htpAB-associated repetitive element, as previously described [5].

Formalin-fixed paraffin-embedded aortic specimens were cut to 3-µm thickness and stained with haematoxylin–eosin–safron, using routine methods. Serial sections were also obtained to perform special stains and immunohistochemical investigations. Special stains, including periodic acid–Schiff, Giemsa, Gram, Grocott–Gomori methenamine silver and Warthin–Starry stains, were used for detection of bacteria and fungi. Immunohistochemical analysis was performed with a monoclonal anti-*C. burnetii* mouse antibody as previously described [6].

**RESULTS**

In our laboratory, IgG titres of >1:800 are regarded as being diagnostic for chronic Q fever [5]. The patient exhibited antibody titres to phase I *C. burnetii* of 1 : 3200, 0 and 1 : 50 for IgG, IgM and IgA, respectively, and antibody titres to phase II *C. burnetii* of 1 : 6400, 0 and 1 : 100 for IgG, IgM and IgA, respectively. Isolation of *C. burnetii* by culture from aortic biopsy specimens was not performed, because the aortic prosthesis removed surgically was fixed in formalin. DNA was extracted from EDTA blood collected at the time of diagnosis using the QiaAmp tissue kit as recommended by the manufacturer (Qiagen). Attempts to detect *C. burnetii* by PCR amplification using primers targeting the htpAB-associated repetitive element gave negative results.

In the aortic wall, mononuclear cell inflammatory infiltrates were rare and focal, and consisted mainly of lymphocytes and macrophages. An important haematoma was also present in the aortic wall. With the immunohistochemical anal-
ysis, bacteria were seen as coarse granular immunopositive material in the macrophage cytoplasm (Fig. 1). C. burnetii could only be visualized within regions of inflammation, as small and focal collections of infected mononuclear cells with a macrophage morphology.

DISCUSSION

We report a new case of C. burnetii infection of an aortic graft. Because Q fever aortitis can be fatal if untreated, an early diagnosis is needed. The diagnosis of Q fever can be established serologically or by cell culture [4,7]. PCR-based methods have also been successfully applied to detection of C. burnetii from excised tissue samples or blood [5,8]. However, the role of the pathologist can be decisive in the recognition of this vasculitis, especially when the microbiologist fails to isolate the causative microorganism. On the other hand, C. burnetii is not observed in tissue specimens when haematoxylin-eosin stain is used, and routine examination of resected aortic tissues is insufficient to establish a diagnosis of Q fever. In this study, we show that immunohistological examination is able to demonstrate the presence of C. burnetii in aortic tissue specimens from a patient with Q fever aortitis. In agreement with other reports [6,9], the organisms were found only in an intracellular location, within the cytoplasm of macrophages of the inflammatory cellular infiltrates.

The failure to detect C. burnetii in the six patients with Q fever aortitis by immunohistochemical analysis in a previous study may result from sampling errors, because the infective process may be confined to a small area, as in the case presented in this study [3]. Finally, as we reported for Q fever endocarditis, the inflammatory reaction associated with Q fever aortitis lacks well-formed granulomas. This observation suggests that patients with chronic Q fever 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 [10–12].

REFERENCES