

Blood Culture–Negative Cardiovascular Infection in a Patient With Multiple Sclerosis

Cléa Melenotte,^{1,2} Ahmed Loukil,^{1,2} Audrey Rico,³ Hubert Lepidi,^{1,4} and Didier Raoult^{1,2}

¹Aix-Marseille Univ, IRD, APHM, MEPHI, Marseille, France, ²IHU-Méditerranée Infection, Marseille, France, ³Service de Neurologie, Assistance Publique des Hôpitaux de Marseille, Marseille, France, and ⁴Service d'Anatomopathologie, Assistance Publique des Hôpitaux de Marseille, Marseille, France

A patient with multiple sclerosis presented with seronegative *C. burnetii* endocarditis diagnosed using *C. burnetii*-specific polymerase chain reaction and fluorescence in situ hybridization on cardiovascular biopsy. This case supports the necessity of a systematic polymerase chain reaction testing of removed cardiac valves because blood culture–negative endocarditis can be pauci-symptomatic, and serological tests can be negative in cases of immunosuppression.

Keywords. *Coxiella burnetii*; endocarditis; immunodepression; Q fever; serology.

A 65-year-old man presented to our hospital for cardiac surgery of an aortic stenosis. His medical history included arterial hypertension, testicular cancer, dyslipidemy, and multiple sclerosis, for which he had been receiving sequential immunosuppressive therapies since 2014. He was first treated with intravenous corticosteroid, which was switched to tecfidera (dimethyle fumarate) in 2015. In 2016, tecfidera was stopped because of persistent lymphopenia (0.7 G/L), teriflunomide was initiated and switched in November 2018 to glatiramer acetate. Associated medications included olmesartan, acetylsalicylate, lansoprazole, and alpha-tocopherol acetate. He used to work in a metal factory and lived in Corsica at the time of presentation. The first transthoracic echocardiography, performed in March 2015, showed a bicuspid aortic remodeled valve. In 2017, a severe aortic stenosis was diagnosed in association with an aneurysm of the ascendant thoracic aorta (40×34 mm); the patient was pauci-symptomatic. In January 2018, an aortic

bioprosthetic valve and an aortic vascular segment I prosthesis were placed. Histological analysis of the surgical material (aortic valve and vascular sample) showed a remodeled valve with fibrosis and calcification (Figure 1A). At the University Hospital of Marseille, La Timone, we systematically screened patients with valvular surgery for bacterial agents responsible for blood culture–negative endocarditis (BCNE). Accordingly, *C. burnetii*-specific polymerase chain reaction (PCR; IS1111 and IS 30a) was positive on the aortic valve and on blood. Anti-*C. burnetii* immunohistochemistry and fluorescence in situ hybridization (FISH) targeting *C. burnetii* was positive on vascular biopsy and negative on the aortic valve (Figure 1B, C). Nonetheless, *C. burnetii* serology (immunofluorescence assay) remained negative from January 2018 to March 2019. Western blot analysis for *C. burnetii* was positive (Figure 1D) [1]. No hypogammaglobulinemia was detected (IgG, 8.5 g/L, IgA, 1.2 g/L, IgM 0.9 g/L). Severe T-cell and B-cell deficits were observed (naïve and memory TCD4, TCD8, memory, and differentiated B cell). Doxycycline associated with hydroxychloroquine was initiated in April 2018 for 24 months, as recommended by the French National Reference Center for Q fever [2]. *C. burnetii* PCR on blood sample became negative 3 months later.

This report highlights 2 major advances. The first is the systematic screening of microbiological agents in patients who have undergone cardiovascular surgery, and the second is the revolutionary impact of PCR on microbiological diagnosis. Microbial agents responsible for blood culture–negative endocarditis should be systematically screened by PCR on fresh removed cardiovascular samples (16S qRT PCR and specific qRT PCR for *Bartonella* sp., *Coxiella burnetii*, and *Tropheryma whippelii*, *S. aureus*, *S. oralis*, *S. mitis*, *S. sanguinis*, *S. gallolyticus*, *E. faecalis*, *E. coli*), especially in immunocompromised patients. Although BCNE may represent 2.5% to 70% of all endocarditis cases, the multimodal diagnosis strategy we used, including systematic blood testing and specific PCR on the valvular biopsy in cases of valvular surgery, made it possible to identify a microbiological agent in 78% of BCNE in a previous study [3, 4]. This strategy is therefore promising for diagnosing BCNE in pauci-symptomatic patients.

In addition, this is the first case of definite *C. burnetii* persistent cardiovascular infection with negative serological testing reported from the French National Reference Center for Q fever. Patients with *C. burnetii* persistent focalized infection and low serological titers (phase I IgG < 800) represent 5% of our cohort. No seronegative *C. burnetii* persistent infection has been reported in the literature [2, 5, 6]. This case supports the paradigm shift in Q fever diagnosis criteria, as neither the serological cutoff phase I IgG > 800 nor positive serology was

Received 18 July 2019; editorial decision 23 September 2019; accepted 30 September 2019.

Correspondence: D. Raoult, MD, PhD, IHU - Méditerranée Infection 19–21 Boulevard, Jean Moulin 13005 Marseille, France (didier.raoult@gmail.com).

Open Forum Infectious Diseases®

© The Author(s) 2019. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com DOI: 10.1093/ofid/ofz429

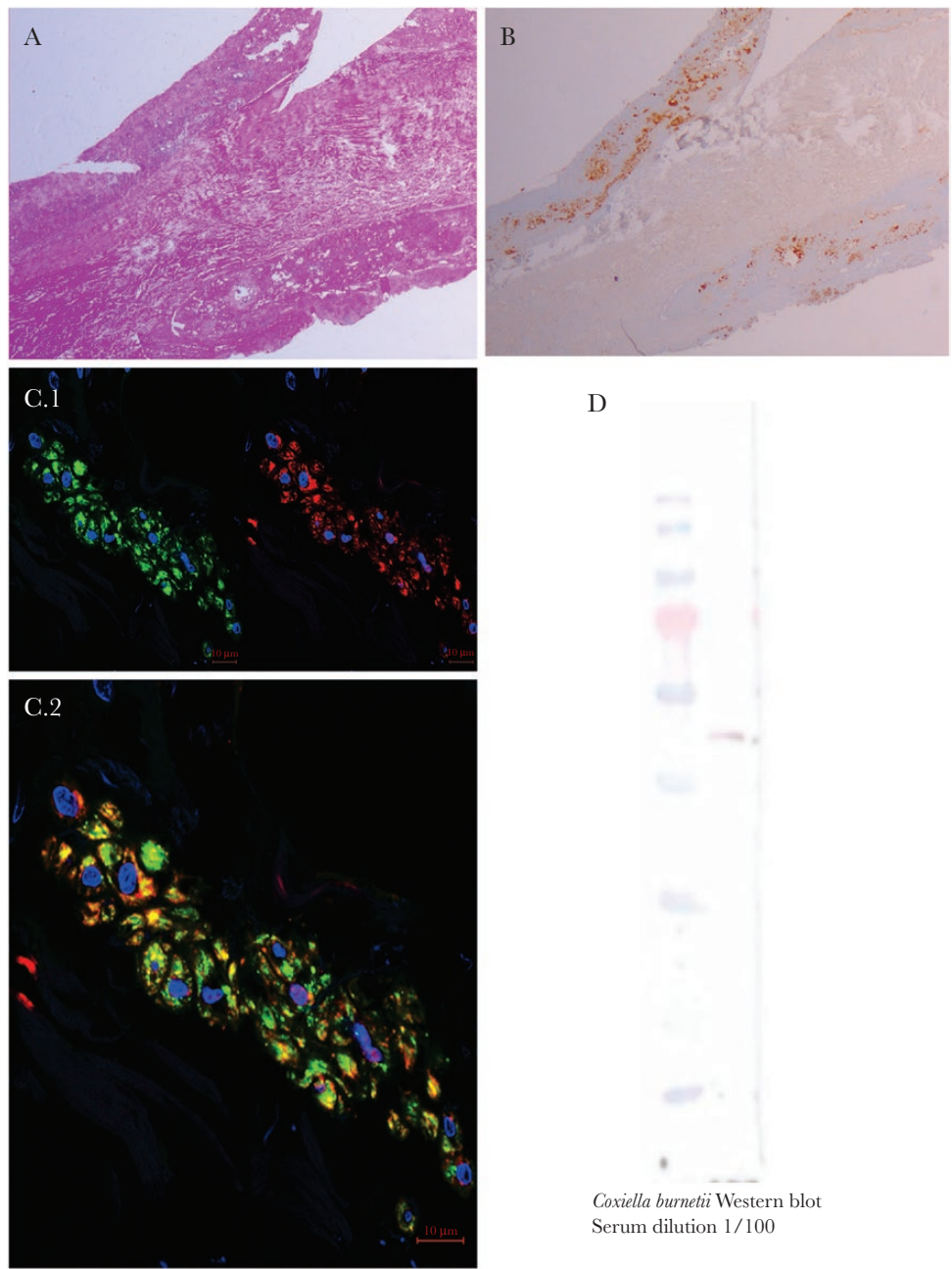


Figure 1. A–C, Aortic vascular tissue. A, Hematoxylin and eosin coloration showed aspecific degenerative lesions of the media. B, Positive anti-*C. burnetii* immunohistochemistry. C1, In situ fluorescence hybridization targeting *C. burnetii* RNA (green), all bacteria DNA (red), and nuclei (blue) (A and C). C2, Positive *C. burnetii* signal (green) and positive universal bacterial probe EUB (red) colocalize as a yellow signal. Confocal microscopy Mx100. D, *C. burnetii* Western blot performed from a peripheral blood sample with a serum dilution at 1/100.

required to diagnose *C. burnetii* endocarditis [7]. This enhances the new definition criteria we use for *C. burnetii* infection, in which microbiological criteria in addition to an organic lesional criteria are required [8]. Here, the microbiological criteria were positive *C. burnetii* specific PCR and immunohistochemistry and FISH on valvular and vascular specimens, respectively, whereas the organic lesion was the damaged aortic valve. This observation highlighted the limit of the serological test and the crucial place of PCR in the diagnosis of Q fever [9]. The patient

was lymphopenic, and the immunomodulatory molecules he received act on B lymphocytes. It is known that teriflunomide blocks de novo pyrimidine synthesis via the selective and reversible inhibition of synthesis of dihydro-orotate dehydrogenase, a key mitochondrial enzyme [10]. This has reduced the proliferation of activated T and B lymphocytes and limited their involvement in the inflammatory process [10]. Nevertheless, no impact of teriflunomide on the immune response to influenza vaccine has been reported, and the influence of teriflunimide on

the humoral response is not well established [11]. Similarly, although glatiramer acetate therapy modulates B cells, the reports are contradictory. First, B cells from glatiramer acetate patients produced significantly more IgM and IgG compared with the B lymphocytes from treatment-naïve patients and healthy donors [12]. Second, glatiramer acetate modifies the humoral response, as Basile et al. identified a shift from Th1 to Th2 in the antibody response [13]. Therefore, the impact of these molecules on the serological response is not clear, but it could have influenced the severe T-cell and B-cell deficits observed here.

This case first evidenced the importance of systematic screening for intracellular bacteria (*Bartonella henselae*, *Bartonella quintana*, *Coxiella burnetii*, *Tropheryma whipplei*) on cardiovascular surgical material to diagnose a negative paucisymptomatic cardiovascular infection in blood culture. In addition, this case also demonstrates the limitations of Q fever serology in diagnosing persistent *C. burnetii* infection, which justifies the need for a microbiological criterion in addition to a lesional criterion [2, 7]. Molecular biology, whether in situ visualization or DNA amplification, has become crucial for the diagnosis of Q fever, particularly in the case of immune disorders, when the serological response is altered.

Acknowledgments

We thank Prof. Michel Drancourt for his proofreading. We thank Elsa Prudent for the transmission of FISH protocols.

Financial support. URMITE, IHU Méditerranée Infection. This work was supported by the French Government under the “Investissements d’avenir” (Investments for the Future) program managed by the Agence Nationale de la Recherche (ANR; fr: National Agency for Research; reference: Méditerranée Infection 10-IAHU-03). This work was also supported by Région Provence Alpes Côte d’Azur and European funding from FEDER

PRIMMI (Fonds Européen de Développement Régional - Plateformes de Recherche et d’Innovation Mutualisées Méditerranée Infection).

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. *J Clin Microbiol* **1998**; 36:1823–34.
2. Melenotte C, Protopopescu C, Million M, et al. Clinical features and complications of *Coxiella burnetii* infections from the French National Reference Center for Q fever. *JAMA Netw Open* **2018**; 1:e181580.
3. Brouqui P, Raoult D. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* **2001**; 14:177–207.
4. Fournier PE, Gouriet F, Casalta JP, et al. Blood culture-negative endocarditis: improving the diagnostic yield using new diagnostic tools. *Medicine (Baltimore)* **2017**; 96:e8392.
5. Edouard S, Million M, Lepidi H, et al. Persistence of DNA in a cured patient and positive culture in cases with low antibody levels bring into question diagnosis of Q fever endocarditis. *J Clin Microbiol* **2013**; 51:3012–7.
6. Melenotte C, Bart G, Kraeber-Bodere F, et al. Isolation of *Coxiella burnetii* from an acromioclavicular infection with low serological titres. *Int J Infect Dis* **2018**; 73:27–9.
7. Raoult D. Q fever: confusion between chronic infection and chronic fatigue. *Clin Infect Dis* **2017**; 65:1054–5.
8. Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus. *J Infect* **2012**; 65:102–8.
9. Fenollar F, Fournier PE, Raoult D. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. *J Clin Microbiol* **2004**; 42:4919–24.
10. Bar-Or A, Pachner A, Menguy-Vacheron F, et al. Teriflunomide and its mechanism of action in multiple sclerosis. *Drugs* **2014**; 74:659–74.
11. Bar-Or A, Freedman MS, Kremenchutzky M, et al. Teriflunomide effect on immune response to influenza vaccine in patients with multiple sclerosis. *Neurology* **2013**; 81:552–8.
12. Ireland SJ, Guzman AA, O’Brien DE, et al. The effect of glatiramer acetate therapy on functional properties of B cells from patients with relapsing-remitting multiple sclerosis. *JAMA Neurol* **2014**; 71:1421–8.
13. Basile E, Gibbs E, Aziz T, Oger J. During 3 years treatment of primary progressive multiple sclerosis with glatiramer acetate, specific antibodies switch from IgG1 to IgG4. *J Neuroimmunol* **2006**; 177:161–6.