Etiological diagnostic of blood culture negative endocarditis

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Infective endocarditis (IE) remained a diagnostic challenge. Etiological diagnosis is critical to select an appropriate treatment, as the fatality rate remains high[1]. The proportion of IE without etiological diagnosis varies from country to country and from different centers in the same country. These variations reflect the used diagnostic criteria, the early use of antibiotics in patients without documentation and the sampling strategy for etiological diagnosis[2]. Moreover the role of epidemiology which as been underestimated appears critical as in a recent series from Algeria zoonotic agents may caused as many as 25% of IE[3].

The diagnostic of IE is currently based on the commonly modified Duke Criteria[4]. Using this strategy requires obtaining high quality echocardiography and blood cultures sampled before antibiotic administration. It also requires testing serologically at the minimum *Coxiella burnetii* and *Bartonella* species. Duke criteria may not be perfectly efficient for blood culture negative IE, as they may be chronic, afebrile and, eventually such as for Q fever, may lack valvular vegetation. Moreover underlying lesions, and specifically aortic bicuspidy, may not be detected by echocardiography. In my own experience in 2 cases with evidence of chronic Q fever, two successive transthoracic echocardiography failed to detect aortic bicuspidy. In such patients without fever, vegetation and obvious underlying valve lesion IE diagnosis remains a real challenge.

Culture of blood is critical for the diagnosis of IE It is clear that 3 samples containing 40 ml of blood, obtained in a 4 hours time is enough to detect usual organisms[2]. This allows starting empiric treatment 4 hours after the first sampling when IE is considered. Extensive blood culture and subculturing is not necessary[5], and HACEK group organism are recovered in the regular 5 days incubation. Specialized culture methods are useful when regular blood cultures fail to recover the etiological agents. We have successfully used the shell vial cell culture assay for this purpose sampling blood or valves of patients with blood culture negative IE[6]. This allows recovering *C. burnetii*, *Bartonella*, *Tropheryma whipplei* and *Brucella*. However this technique is restricted to specialized laboratories.

Blood culture negative IE (BCNIE) is defined as an endocarditis without etiology after 3 blood samples inoculated on standard medium. The causative agents of BCNIE are fastidious organisms (zoonotic agents and fungi) *Streptococci* in patients who received previous antibiotic treatment and infection of the right heart and specifically on pace makers.

The diagnostic strategy of BCNIE starts by serological testing of fastidious agents[2]. At the minimum *Coxiella burnetii* and *Bartonella* should be tested. A single serum is sufficient, as endocarditis is a chronic disease associated with high level IgG antibodies. Q fever endocarditis is associated with antibody titers to *C. burnetii* phase I above 800 and *Bartonella* endocarditis to high levels ($\geq$ 800) of IgG[2]. Patients with Q fever may have cross-reacting antibodies to *Bartonella*. Other organisms may be tested depending on the epidemiological situation including *Brucella*, *Legionella pneumophila* and *Aspergillus*[2]. Antibodies to *Chlamydia* usually result from cross-reactions with *Bartonella*, and there are few evidences that it can cause IE[7].

Detection by PCR of organisms in the blood is a promising technique[8]. Real time PCR has been successfully developed for the diagnosis of *Bartonella* IE[9] and Q fever[10]. A preliminary study using broad spectrum primers was also performed[11] and a PCR amplification of blood for the diagnosis of Whipple's diseases has also been proposed[12]. However currently these techniques frequently lack specificity. PCR when performed on the valve has been recently reported in several studies. The use of universal primers amplifying any bacteria (based on 16S rRNA) or fungi (based on 18S rRNA) has been reported[13]. The detected microorganisms are mainly Streptococcus in patients with previous antibiotics treatment, and fastidious bacteria including *Granulicatella*, *Abiotrophia*, *Bartonella, Coxiella burnetii* and *Tropheryma whipplei.*

DNA from the causative agent can persist months to years after clinical cure and the link between the current IE episode and the amplified DNA need to be carefully checked[14]. Moreover PCR can easily be contaminated and controls are necessary. Personally I recommend not using positive controls that may be confused with the causative agents, as carry over contamination is common. DNA from a microorganism very unlikely causing IE may be a good positive control. Negative controls are critical. They should be placed any 3 to 7 tested samples and be negative. Any positive amplicon should be sequenced to identify the causative agent. Some sequences usually result from contamination and are easily recognized such as DNA commonly found in the water *(Pseudomonas)* or the reagents *(E. coli)*. A similar sequence found in the same round of PCR in a sample from another patient may result from contamination. In the other hand a sequence found for the first time in a laboratory reflect usually a true positive result. When the results have a low predictive value, amplification of a second gene is critical to confirm the etiological diagnosis.

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Histological examination of the valve may be useful[15]. Regular staining allows to identify IE and to recognize pseudo endocarditis (myxoma, fibroelastoma or rheumatoid nodules) and non infectious endocarditis (marastic endocarditis, inflammation in degenerative valvular lesions, rheumatic endocarditis and Libman-Sacks endocarditis). Immunohistochemistry may allow to specifically identifying to causative agent including *Bartonella*, *Coxiella burnetii* and *Tropheryma whipplei*. This technique makes it possible to test retrospectively even several years after, the stored tissues. In a very unique case we have been able to confirm the presence of *T. whipplei* in an original tissue sample of the index case of Whipple's disease described by G. Whipple nearly one hundred years before[16].

Among agents causing BCNIE *Coxiella burnetii* and *Bartonella* are the more common[17]. Q fever is common in Europe including Spain and may cause 3 to 10% of IE Its incidence is estimated at 1/1 million inhabitants per year in France, Israel and Switzerland. It is much more rare in Northern Europe and the United Kingdom[18]. Q fever IE is indolent and chronic. It may be afebrile and only 40% may have valvular vegetations at the echocardiography. Currently in countries like Spain, Switzerland, France or Israel where the disease is actively tested the clinical spectrum have changed. Significant decreases were found in the prevalence of heart failure, hepatomegaly, inflammatory syndrome, leukopenia ad abnormal liver function tests. This probably result of a reduction in the delay before diagnosis of the disease and the use of novel effective antibiotic regimens[19].

Bartonella endocarditis, as reported here by J.A. Oteo et al. in this issue[20], is also commonly found as a cause of BCNIE. The diagnosis is usually based on serology or PCR of the valve. *B. quintana* causes 70% of *Bartonella* IE in large series. The prevalence of *Bartonella* IE varies from place to place and increase from North to South in Europe. It was reported recently that the prevalence of *Bartonella* among IE was $\leq$ 1% in Northern Europe, 1% in England, 3% in France and Germany and $\geq$ 10% in Tunisia and Algeria (3;7). Most of the cases found in Northern Africa were caused by *B. quintana*. However, in the present study by J.A. Oteo (20) the causative agent is *B. henselae*.

The other causes of BCNIE are streptococci, *T. whipplei* and fungi that are essential to diagnose to benefit from a specific treatment. The epidemiology of the causative agent widely varies from countries to countries. In rural countries zoonotic agents are more commonly found[3]. BCNIE are more common in developing countries. For years this was considered as a consequence of poor diagnostic tests. Recently we showed in Algeria that this was rather due to fastidious zoonotic agents such as *Bartonella*, *Coxiella burnetii* or *Brucella*[3]. In rich countries pace maker associated BCNIE are increasingly common.

Even, using all available techniques, IE causative agent may remains ignored. In a recent standardized study we found that the causative agent of 30 out of 427 endocarditis remains unknown[2]. Among unresolved cases some were related to previous antibiotic treatment on right side IE. However 5 patients that benefited from an exhaustive testing strategy including valve testing by PCR remain unresolved.

In conclusion, the diagnostic of IE has largely benefited from the standardization of the diagnostic score[4] and by the use of serology[2] and PCR[8]. A standardization of the diagnostic procedures may help in the future the identification of IE etiological agents.

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