

Letter to the Editor

Real-Time Polymerase Chain Reaction Seems Not to Be Superior to Blood Cultures in Anticipating Valve Sterilization in Infective Endocarditis

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The diagnosis of infective endocarditis remains problematic, mainly because of the inherent limitations of blood cultures, such as delays in results and low sensitivity. Molecular techniques, notably polymerase chain reaction (PCR), can detect pathogens in surgically excised vegetations and in the blood of such patients.¹⁻³ They could also be useful during the treatment of endocarditis, as real-time PCR could monitor the bacterial load in blood⁴ and probably in vegetations. Relapse of fever could be ascribed to a complication of endocarditis *per se* or to another entity (e.g. thromboembolism). The concept is that real-time PCR is a sensitive tool and, even if the bacterial load is too small to be detected by blood cultures or is inhibited by antibiotics, it could detect bacteremia. So far, there are no convincing data to support the position that molecular methods exceed the sensitivity of cultures.⁵ Nevertheless, if this hypothesis proved to be true, it would have serious implications in the management of infective endocarditis, with regard to the duration of treatment, earlier recognition of complications or treatment failures.

During experiments in a rabbit mod-

el of enterococcal endocarditis, regarding the efficacy of daptomycin, we conducted a parallel study based on this hypothesis. After left catheterization (day 0) and inoculation of 10⁸ CFUs of a clinical strain of *Enterococcus faecium* (day 2), daptomycin was initiated for five days (4-8). On day 9 animals were sacrificed, vegetations excised and quantitatively cultivated. Before inoculation, treatment initiation and sacrifice, blood samples were drawn for culture in BACTEC (Becton Dickinson) bottles and PCR. Identification of *Enterococcus* was performed by conventional methods and ID panels (VI-TEK 2, bioMérieux). Blood samples for PCR were collected in 3 ml EDTA tubes and stored at -20°C. Hemoglobin was removed and finally DNA was extracted from 200 µl samples using the High Pure PCR Template Preparation Kit (Roche). The extract was eluted in 100 µl of elution buffer. The LightCycler *Enterococcus* Kit MGRADE (Roche), designed for use on the LightCycler platform (version 1.5), was employed. 5 µl of the extracted DNA were used in a total of 20 µl reaction mixture. A standard curve of *E. faecium* DNA, consisting of seven 10-fold dilutions

of a stock solution of 10^8 CFU (10^7 CFU equivalents/PCR), was included in each run for the automatic calculation of bacterial DNA load. In order to determine the reproducibility of the bacterial DNA load, 10 culture-negative whole-blood samples were spiked with 500 CFU (50 CFU equivalents/PCR) of *E. faecium*, followed by DNA isolation. Analytical sensitivity was determined to be less than 10 CFU equivalents/PCR. PCR amplification results were available for all blood samples (n=30 paired samples). No discrepancies were observed among paired blood samples. No false positive results were observed.

All blood cultures on day 4 were positive and sterile on days 2 and 9. No excised vegetation was sterile (mean CFU \pm sd: $5.75 \pm 0.89 \text{ Log}_{10}$ CFU/g). Results of real-time PCR were positive during proved bacteremia ($3503 \pm 1.2 \text{ Log}_{10}$ copies/reaction), but negative when cultures turned sterile. No correlation was found between bacterial DNA load on day four and final bacterial load on vegetations.

Our results suggest that real-time PCR is not sensitive enough in the case of animal enterococcal endocarditis to detect bacteremia when blood cultures have turned negative. Thus, its utility in predicting valve sterilization is improbable. A possible explanation is that the infection was strictly confined under

treatment without bacterial shedding in the circulation, or that the sensitivity is too low because of the small volume of blood used in DNA isolation. On the other hand, DNA load before initiation of treatment may not be a predictor of final outcome. Further studies are needed to determine the optimal molecular methodology needed in the diagnosis and treatment of infective endocarditis.

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