BRIEF REPORT

Evaluation of a Multiplex Real-Time PCR Assay for Detecting Pathogens in Cardiac Valve Tissue in Patients With Endocarditis
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With a novel real-time multiplex polymerase chain reaction test, the LightCycler SeptiFast® test, 25 bacterial and fungal species can be identified directly in blood. The SeptiFast® test has been used for rapid etiologic diagnosis in infectious endocarditis using blood samples but has not been evaluated directly on cardiac vegetations in patients being treated for infectious endocarditis. We prospectively analyzed 15 samples of heart valve tissue with active infectious endocarditis using the SeptiFast® test and compared the test’s sensitivity with that of blood culture, valve tissue culture, and the SeptiFast® test in blood. The sensitivity of the SeptiFast test in heart valve tissue was 100%. The test results confirmed the diagnosis obtained using blood culture in 13 cases and identified the pathogen in 2 cases where blood culture tested negative. The sensitivity of the SeptiFast® test in heart valve tissue was greater than that obtained with conventional culture of vegetations or with the SeptiFast test in blood.

Key words: Endocarditis. Polymerase chain reaction.

INTRODUCTION

The culture of heart valve tissue in patients being treated for active infective endocarditis (IE) can result in a false positive due to contamination of the sample or in a false negative as a consequence of antifungal therapy or because the microorganisms in question can not be cultured.1,2

Polymerase chain reaction (PCR) is a more sensitive technique than conventional culture methods for the amplification and detection of microbial deoxyribonucleic acid (DNA) in heart valve tissue and its reliability has been verified in IE and in the identification of new pathogenic agents.2,6 The demonstration of the presence of microbial DNA in heart valve tissue has been proposed to be a major diagnostic criterion in IE.4 At the present time, the molecular analysis of heart valve tissue or of the embolic material is recommended in IE when the blood culture is negative.7

A test has recently been introduced for the molecular diagnosis of septicemia by means of multiplex real-time PCR capable of identifying, in a
1.5-mL blood sample, bacterial or fungal DNA of the 25 pathogens that most frequently cause septicemia (LightCycler SeptiFast® test, Roche Diagnostics, Penzberg, Germany) (Table 1).

The use of SeptiFast® in blood has produced promising results in sepsis and febrile neutropenia, and has enabled the determination of the etiologic diagnosis in IE with negative blood culture, although there are no data concerning its application in heart valve tissue. This report presents our preliminary experience with the SeptiFast® test for the analysis of vegetations in active IE.

**METHODS**

Between August 2007 and September 2009, we studied 15 patients (11 men and 4 women) with active IE who had undergone heart valve surgery. The mean age was 70.2 years (range, 57 to 82 years). All the patients had definite IE according to the modified Duke criteria. The IE was located over left native valve (6 cases), valve prosthesis (8 cases) and tricuspid valve (1 case).

Prior to their being referred for surgery, blood samples had been collected in other hospital services, and were found to be positive in 13 cases and negative in two. All the patients had received antinfective therapy between 24 hours and 82 days earlier, but had not completed the treatment properly.

During the preoperative evaluation, carried out between one and 24 days after the blood culture, blood was collected for study using the SeptiFast® test according to the procedure described elsewhere. During the operation, a sample of heart valve tissue with vegetations or vegetations alone, in the case of a prosthesis, was introduced into a DNA-free container with no additives.

The tissue DNA was obtained by means of mechanical lysis (MagNa Lyser, Roche Diagnostics, Penzberg, Germany) and subsequent extraction (Magna Pure Compact, Roche Diagnostics, Penzberg, Germany). Molecular analysis was carried out using the SeptiFast® system according to the manufacturer's instructions; the microbiologist did not know the results of the previous blood cultures. The mean time employed in the molecular analysis of the tissue from the arrival of the sample in the laboratory until the reception of the results was 3.57 hours.

One tissue fragment was cultured according to the conventional method in blood agar, chocolate agar (5%, CO2), Sabouraud agar, Schaedler agar and thioglycolate broth. The identification of the isolated microorganisms was carried out with the Vitek 2 system (BioMérieux, Nancy l'Etoile, France).

**RESULTS**

The results of the blood cultures performed before the referral of patients for surgery are shown in Table 2. The 2 patients with negative results had received intermittent oral and intravenous antibiotic therapy in the centers that had referred them for more than four weeks prior to collection of the samples for blood culture. The SeptiFast® test in blood was positive in eight cases and negative in seven (Table 2).

The heart valve tissue culture was positive in 5 cases and negative in 10 (Table 2). The SeptiFast® test was positive in the heart valve tissue of all the patients, results that coincided with the findings in the blood cultures and with SeptiFast® in blood. We observed a discrepancy between the culture of the vegetation and SeptiFast® in heart valve tissue in one case (case no.10), which was interpreted as a possible contamination of the cultured sample (Table 2).

**DISCUSSION**

Previous studies have shown that the amplification of the 16S gene of ribosomal ribonucleic acid (rRNA)
by means of PCR using broad-range primers is a more sensitive technique than blood culture and heart valve tissue culture for the etiologic diagnosis of IE since it enables universal detection, although, because of its complexity, its systematic use is restricted to certain laboratories.3,4

The SeptiFast® test amplifies the internal transcribed spacer (ITS) in the rRNA gene region and its advantage is based on the simplicity and the shorter response time; however, in contrast to the universal primers, SeptiFast® only detects the DNA of those microorganisms whose target sequence is included, and it is not able to identify all of the germs that can cause IE.

In the blood of patients with IE, the SeptiFast® test has shown a sensitivity similar to that of blood culture for streptococci, enterococci and Staphylococcus aureus.13 In cases of IE treated with antinfective agents and with a negative blood culture, SeptiFast® is capable of detecting bacterial DNA in the bloodstream,13 as we were able to confirm in our series (cases nos. 3 and 4). In patients with active IE, it is also possible to obtain a positive blood culture and, days later, a negative SeptiFast® test in blood despite the coexistence of vegetations with bacterial DNA. The latter phenomenon was observed in our series (cases nos. 6 to 9, 14 and 15) when the patient had received antibiotic therapy for more than five days during the period between the blood culture and the SeptiFast® test.

SeptiFast® has been reported to exhibit a lower sensitivity to coagulase-negative staphylococci (CoNS) as compared to blood culture.10,12,13 This could be due to a low concentration of bacterial DNA in blood secondary to the slow release of DNA from the vegetations into the bloodstream or to DNA inactivation.12

Moreover, SeptiFast® has been designed for the diagnosis of sepsis and has established a relatively high cut-off point for sensitivity for the DNA of CoNS and streptococci and, thus, low concentrations were not considered to be positive. The purpose of this software program is to filter the cases of contaminant-related transient bacteremia related to invasive techniques (intravenous catheters, probes) routinely employed in critical care units.11 In our experience, we consider that patients with heart valve prostheses, in whom CoNS are the major etiologic agents in early postoperative IE, each case should be evaluated jointly by the clinician and the microbiologist to differentiate between a contaminant and a pathogen. In this respect, our patients nos 5, 10, and 11 developed early endocarditis over their prosthesis, and SeptiFast® in blood detected CoNS DNA, but the identification software interpreted it as possible contamination, whereas the blood culture and SeptiFast® test in heart valve tissue were positive.

The sensitivity of the SeptiFast® test in vegetations was 100%, whereas that of the culture of heart valve tissue was 30.7%, similar to that observed with other molecular techniques.2-4 In our series, we were able to confirm, as did other authors,3 that the sensitivity of PCR is not affected by the duration of antinfective therapy administered prior to surgery. This circumstance

<table>
<thead>
<tr>
<th>Case</th>
<th>Daysa</th>
<th>Blood Cultureb</th>
<th>SeptiFast® in Bloodc</th>
<th>Heart Valve Culture</th>
<th>SeptiFast® in Heart Valve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
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<td>1</td>
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<tr>
<td>3</td>
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<td>–</td>
<td>E faecalis</td>
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<tr>
<td>4</td>
<td>35</td>
<td>–</td>
<td>E coli</td>
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<tr>
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<td>CoNS</td>
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<td>CoNS</td>
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<tr>
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<tr>
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<td>–</td>
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<td>13</td>
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<tr>
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<tr>
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<td>S aureus</td>
<td>–</td>
<td>–</td>
<td>S aureus</td>
</tr>
</tbody>
</table>

[a] Total number of days of continuous or intermittent antibiotic therapy prior to surgery.
[b] Blood samples were collected prior to referral of the patients for surgical treatment.
[c] The sample for SeptiFast® was collected between 1 and 24 days after blood culture.
[d] The sample was considered to be contaminated.

−: negative; CoNS: coagulase-negative staphylococci; E: enterococcus; S: streptococcus.
may be due to the fact that the clearance of the DNA of the viable or nonviable bacteria present in the vegetation is slow, and bacterial DNA can persist several years after completion of the antimicrobial therapy.¹⁴

Our results confirm that the application of the SeptiFast® test for the study of heart valve tissue in IE is rapid, sensitive and easily reproducible. Given that it is a molecular technique, SeptiFast® does not identify viable germs, but microbial DNA. As we indicated above, the SeptiFast® test is designed to identify a wide group of bacterial and fungal species, but does not detect other possible pathogens in IE, such as the HACEK group, Gemella, Coxiella and Bartonella and, thus, a negative SeptiFast® test does not rule out IE.

REFERENCES