BRIEF REPORT

Evaluation of a Multiplex Real-Time PCR Assay for Detecting Pathogens in Cardiac Valve Tissue in Patients With Endocarditis

Ángel L. Fernández,[a] Eduardo Varela,[b] Lucía Martínez,[b] Amparo Martínez,[c] Juan Sierra,[a] José R. González-Juanatey,[c] and Benito Regueiro[b]

[a]Servicio de Cirugía Cardiaca, Hospital Clínico Universitario, Santiago de Compostela, A Coruña, Spain
[b]Servicio de Microbiología, Hospital Clínico Universitario, Santiago de Compostela, A Coruña, Spain
[c]Servicio de Cardiología, Hospital Clínico Universitario, Santiago de Compostela, A Coruña, Spain

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.*

Evaluación de una PCR multiplex en tiempo real para la detección de patógenos en el tejido valvular de pacientes con endocarditis

Un nuevo test multiplex basado en una reacción en cadena de polimerasa en tiempo real LightCycler SeptiFast® permite la identificación de 25 especies bacterianas y fúngicas directamente desde la sangre. El test SeptiFast® ha sido utilizado para el diagnóstico etiológico rápido de la endocarditis infecciosa, pero no ha sido ensayado directamente sobre las vegetaciones cardiacas de pacientes intervenidos por endocarditis infecciosa. Se realizó un estudio prospectivo para analizar 15 muestras de tejido valvular con endocarditis infecciosa activa utilizando SeptiFast® y comparando su sensibilidad con el hemocultivo, el cultivo del tejido valvular y el SeptiFast® en sangre. La sensibilidad del SeptiFast® del tejido valvular fue del 100%, confirmó el diagnóstico obtenido mediante hemocultivo en 13 casos y proporcionó el diagnóstico etiológico en 2 casos con hemocultivo negativo. La sensibilidad de SeptiFast en el tejido valvular fue superior al cultivo convencional de las vegetaciones y al SeptiFast en sangre.

Palabras clave: *Endocarditis. Reacción en cadena de la polimerasa.*

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 antiinfective therapy or because the microorganisms in question can not be cultured.[1,2]

Correspondence: Dr. A.L. Fernández.
Servicio de Cirugía Cardiaca. Hospital Clínico Universitario.
Travesía Choupana, s/n. 15706 Santiago de Compostela.
A Coruña. Spain.
E-mail: alfg@secardiologia.es

Received September 23, 2009.
Accepted for publication November 25, 2009.

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

TABLE 1. **Microorganisms Detectable by Means of LightCycler SeptiFast®[9] Multiplex PCR**

Gram-Negative	Gram-Positive	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	*CoNS*	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus spp.**	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Aspergillus fumigatus*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Candida krusei*
Acinectobacter baumannii		
Stenotrophomonas maltophilia		

CoNS indicates coagulase-negative staphylococci identifiable by the SeptiFast® test (they include: *S epidermidis, S haemolyticus, S hominis, S pasteuri, S warneri, S cohnii, S lugdunensis, S capitis, S caprae, S saprophyticus, and S xylosus)*; PCR, polymerase chain reaction.
*The streptococci identifiable by the SeptiFast® test include: *S agalactiae, S pyogenes, S anginosus, S bovis, S constellatus, S cristatus, S gordonii, S intermedius, S milleri, S mitis, S. mutans, S oralis, S parasanguinis, S salivarius, S sanguinis, S thermophilus, S vestibularis, and S viridans.*

1.5-mL blood sample, bacterial or fungal DNA of the 25 pathogens that most frequently cause septicemia[8] (LightCycler SeptiFast® test, Roche Diagnostics, Penzberg, Germany) (Table 1).[9]

The use of SeptiFast® in blood has produced promising results in sepsis[10,11] and febrile neutropenia,[12] and has enabled the determination of the etiologic diagnosis in IE with negative blood culture,[9] 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.[13] 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 antiinfective 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.[8,10] 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;[8] 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%, CO_2), 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)

TABLE 2. **Results of Cultures and of Molecular Studies in Blood and Heart Valve Tissue**

Case	Days[a]	Blood Culture[b]	SeptiFast® in Blood[c]	Heart Valve Culture	SeptiFast® in Heart Valve
1	15	*Streptococcus spp.*	*Streptococcus spp*	–	*Streptococcus spp*
2	1	*S aureus*	*S aureus*	*S aureus*	*S aureus*
3	82	–	*E faecalis*	–	*E faecalis*
4	35	–	*E coli*	–	*E coli*
5	9	*CoNS*	*CoNS*	–	*CoNS*
6	18	*S intermedius*	–	–	*Streptococcus spp*
7	11	*S agalactiae*	–	–	*Streptococcus spp*
8	39	*E faecalis*	–	–	*E faecalis*
9	13	*S aureus*	–	–	*S aureus*
10	10	*S epidermidis*	*CoNS*	*S. mitis*[d]	*CoNS*
11	7	*S epidermidis*	*CoNS*	*S. epidermidis*	*CoNS*
12	4	*S aureus*	*S. aureus*	*S. aureus*	*S aureus*
13	3	*S aureus*	*S. aureus*	*S aureus*	*S aureus*
14	54	*S oralis*	–	–	*Streptococcus spp*
15	25	*S aureus*	–	–	*S aureus*

[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*.

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 antiinfective 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 antiinfective therapy administered prior to surgery. This circumstance

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 antiinfective 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

1. Muñoz P, Bouza E, Marín M, Alcalá L, Rodríguez Créixems M, Valerio M, et al. Heart valves should not be routinely cultured. J Clin Microbiol. 2008;46:2897-901.
2. Breitkopf C, Hammel D, Scheld HH, Peters G, Becker K. Impact of a molecular approach to improve the microbiological diagnosis of infective heart valve endocarditis. Circulation. 2005;111:1415-21.
3. Voldstedlund M, Pedersen LN, Baandrup U, Klaaborg KE, Fuursted K. Broad-range PCR and sequencing in routine diagnosis of infective endocarditis. APMIS. 2008;116: 190-8.
4. Marín M, Muñoz P, Sánchez M, Del Rosal M, Alcalá L, Rodríguez-Créixems M, et al. Molecular diagnosis of infective endocarditis by real-time broad-range polymerase Chain reaction (PCR) and sequencing directly from heart valve tissue. Medicine. 2007;86:195-202.
5. Lang S, Watkin RW, Lambert PA, Bonser RS, Littler WA, Elliott TS. Evaluation of PCR in the molecular diagnosis of endocarditis. J Infect. 2004;48:269-75.
6. Kotilainen P, Heiro M, Jalava J, Rantakokko V, Nikoskelainen J, Nikkari S, et al. Aetiological diagnosis of infective endocarditis by direct amplification of rARN genes from surgically removed valve tissue. An 11-year experience in a Finnish teaching hospital. Ann Med. 2006;38:263-73.
7. Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis. Eur Heart J. 2009 [Epub ahead of print].
8. Lehmann LE, Hunfeld KP, Emrich T, Haberhausen G, Wissing H, Hoeft A, et al. A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. Med Microbiol Immunol. 2008;197:313-24.
9. Casalta JP, Gouriet F, Roux V, Thuny F, Habibi G, Raoult D. Evaluation of the LightCycler® SeptiFast test in the rapid etiologic diagnostic of infectious endocarditis. Eur J Clin Microbiol Infect Dis. 2009;28:569-73.
10. Lehmann L, Álvarez J, Hunfeld K, Goblio A, Kost G, Louis R, et al. Potencial clinical utility of PCR in microbiological testing for sepsis. Crit Care Med. 2009 [Epub ahead of print].
11. Westh H, Lisby G, Breysse F, Böddinghaus B, Chomarat M, Gant V, et al. Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis. Clin Microbiol Infect. 2009;15:544-51.
12. Lilienfeld-Toal M, Lehmann LE, Raadts AD, Hahn-Ast C, Orlopp KS, Marklein G, et al. Utility of a commercially available multiplex real-time PCR assay to detect bacterial and fungal pathogens in febrile neutropenia. J Clin Microbiol. 2009;47:2405-10.
13. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633-8.
14. Rovery C, Greub G, Lepidi H, Casalta JP, Habib G, Collart F, et al. PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. J Clin Microbiol. 2005;43:163-7.