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Mini Review

Role of New Microbiological Techniques in the Diagnosis of Infective Endocarditis

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Abstract

Infective endocarditis is a life-threatening disease caused by bacterial infection of the endothelium and cardiac valves, either native or prosthetic. In the present work the role of the new microbiological techniques (techniques of detection and amplification of the subunit 16 ribosomal sRNA by means of the chain reaction of the polymerase in blood or tissue, fluorescent in situ hybridization, and matrix-assisted laser is reviewed desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in the diagnosis of infective endocarditis. Infective endocarditis (IE) is a life-threatening disease caused by septic vegetations and inflammatory foci on the surface of the endothelium and the valves. Although most of endocarditis cases are community-acquired, healthcare-associated endocarditis is increasing and now accounts for approximately one third of IE. Risk factors include the presence of a prosthetic heart valve, structural or congenital heart disease, intravenous drug use and a recent history of invasive procedures. Diagnosis is made using the Duke criteria, which include clinical, laboratory and echocardiographic findings. IE associates high morbidity and a mortality rate close to 25% in the year following diagnosis, thus early diagnosis and adequate treatment is fundamental in its evolution [1].

Most IE, regardless of valve type, native or prosthetic, are due to gram-positive cocci. Currently Staphylococcus aureus is the microorganism most frequently involved, causes 25% to 30% of cases, followed by viridans group Streptococci (20-25%), coagulase negative Staphylococci (11%) and Enterococcus faecalis 10% [2]. Gram-negative bacilli make up 5% of cases and include organisms from the HACEK (Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, and Kingella species) group, Enterobacteriaceae and nonfermenting Gram-negative bacilli. Finally, there is a series of microorganisms difficult to grow in conventional media that can cause IE, the most common being Coxiella Burnetii, Bartonella spp and Tropheryma whipplei and rare cases of IE caused by fungi [3,4]. With traditional microbiological methods, up to one third of IE remain without an etiological diagnosis [5,6]. This is usually due to two factors: the prior taking of antibiotics, which causes a lower yield of blood cultures, and the local prevalence of fastidious microorganisms that are difficult to isolate with conventional microbiological techniques or uncultivable or challenging to cultivate organisms in routine methods [7]. In recent years, various identification techniques have been developed, both in blood cultures and valvular tissue, which entail shorter times and greater diagnostic precision [8], thus reducing the percentage of blood culture negative IE (BCNE)

Introduction

The polymerase chain reaction (PCR) is the most commonly used. A useful target-gene is the 16S ribosomal RNA (rRNA), which is composed of highly conserved regions and variable regions that allow for design of either broad-range PCR primers or genus-or species-specific primers Sequencing of the 16S rRNA gene allows culture-independent phylogenetic classification of many bacteria, and to date is the bacterial gene with the most available sequences in public databases.

PCR-based techniques in blood samples from patients with IE

The application of molecular techniques directly in whole blood samples offers the possibility of identifying the responsible etiological agent in a short period of time. There are few studies that use these techniques on patients with IE. In most cases, patients with sepsis were evaluated using the standard blood culture as a comparator, whose sensitivity is not adequate to be considered the gold standard [9]. This extraordinarily sensitive method has a theoretical detection threshold of only 1 to 10 microorganisms. Its main limitations are false positive results: risk of attributing an IE to the detection of contaminating microorganisms and its inability to detect the real viability of the isolations. There are commercialized techniques that allow the detection of various bacteria and fungi in direct blood samples in real time. In this regard, Casalta et al. [9], compare the sensitivity of the blood culture to a commercially available wide-range polymerase chain reaction (PCR) assay [(SeptiFast (Roche Diagnostics)] for the detection in blood of 19 bacterial species and six fungal species in 63 patients with infectious endocarditis (IE).

They proved that it was not more sensitive for normal organisms, although in 20 patients presenting IE with negative blood cultures caused by previous antibiotic therapy, the trial detected three cases with Streptococcus gallolyticus, Staphylococcus aureus and Enterococcus faecalis, respectively. However, the test does not include the organisms of the HACEK group. The analytical specificity of the test was 100%. More recently, Marco et al. [10] publishes a review on the different molecular methods currently marketed to make a direct diagnosis using blood samples in patients with septicaemia [11]. It concludes that, although these techniques have the potential to reduce the necessary time to reach a final microbiological diagnosis, it does not replace the one obtained with the usual blood cultures. Both methods are currently considered complementary and studies are needed to assess the possible impact they may have on the management of these patients. In addition, other aspects such as the number of samples to be analysed, their frequency or the interpretation of the results from a clinical and microbiological point of view should be considered.

PCR-based techniques for the diagnosis of IE in cardiac valvular tissue

PCR on valvular tissue may offer a higher sensitivity than that obtained in blood cultures, due to the greater abundance of bacterial DNA in the valvular tissue than in blood. For this reason, the application of PCR to explanted valves may increase the sensitivity of the Duke criteria in establishing the diagnosis of definite IE. Moter et al. [11] in 2010 issues a compilation of studies that investigate the impact of PCR-based methods using heart valve samples for the microbiological diagnosis of IE. The results were contrasted with the data obtained by routine blood culture procedures. A total of 481 IE cases from 10 studies were reviewed, including 110 (22.9%) of BCNE cases. In the BCNE group, PCR identified microorganisms in 47 cases, which correspond to 42.7% of BCNE and 9.8% of the total cases investigated. All these studies recommended the application of molecular techniques for the microbiological diagnosis of BCNE and possible IE. However, it should be emphasized that the data from these studies are complex and require careful interpretation. There are several limitations:

1. The results are influenced by differences in regional epidemiology and the inclusion criteria in the different studies.
2. The differences between laboratories and the lack of standardization in PCR-based methods complicate the interpretation of the results.
3. The identification of false positive results. It has been proven that bacterial DNA can persist in cardiac valves under antibiotic therapy and after IE is cured. The viability of these microorganisms is not clear, and the detection of bacterial DNA should be interpreted with caution.

In recent years, to improve the sensitivity and minimize the limitations of these molecular methods, various studies have been carried out; Brinkman CL et al. [12], evaluated the combined use of the mass spectrometry technique and a wide-spectrum PCR in 83 paraffin-fixed heart valves of subjects with endocarditis who had positive valve disease and/or blood cultures. In 55% of cases, there was concordant microbiology at the level of genus/species or group of organisms, 11% positivity with discordant microbiology and 34% without detection. It also detected all the antimicrobial resistance encoded by mec A or van A/B and identified a case of Tropheryma whipplei endocarditis previously unrecognized [13]. The association of both techniques has the potential to reduce the response time compared to the standard culture and in addition to the detection and classification of organisms, the selected antimicrobial resistance is also defined. All several studies conducted with different commercial systems of real-time broad-spectrum PCR that compare the results with conventional valve cultures show a significant difference in the rate of detection of pathogens in favour of the molecular methods, with a high specificity (77% to 100%) and high PPV 100% and NPV (85-90%) [8,14-16].

In order to improve the efficacy of broad-spectrum PCR techniques, new modalities are being used, such as the use of a multiple PCR associated with a nested one, which demonstrates its effectiveness particularly in cases involving a limited number of bacteria, in BCNE and other contexts that involved bacterial species that were not susceptible to identification by phenotypic research [17]. When considering molecular testing of cardiac valves, it should be noted that organism-specific PCR assays often demonstrate superior sensitivity compared to wide-range PCR [18]. For the diagnosis of Bartonella endocarditis, the Bartonella specific PCR applied to the heart valve tissue was positive in 92% (48/52) of the cases, while the broad-spectrum PCR identified Bartonella in the valvular tissue in only 60% (21/35) of the cases [19]. The group led by Didier Raoult demonstrates this in a study conducted in 283 patients with BCNE by adding a specific PCR to the systematic PCR test already used in patients with BCNE, increasing the diagnostic efficiency by 24.3%, mainly by detecting enterococci and streptococci that had not been detected by other diagnostic methods, but also agents that required specific management such as Mycoplasma hominis and Tropheryma whipplei [20].

Diagnosis Based on Fluorescent in situ Hybridization

Fluorescence in situ hybridization (FISH) is a molecular technique that uses fluorescently labelled probes to detect RNA or DNA. Fluorescent probes are applied to fixed samples, either smears or sections of tissue on slides. They penetrate in morphologically intact microorganisms and hybridize specifically at their target sites on highly abundant ribosomes. Therefore, FISH allows the independent identification of the culture and the simultaneous visualization of bacteria. Although FISH techniques could potentially be useful to improve the diagnostic yield in EIs, there are not enough contrasted studies that allow this technique to be incorporated into routine clinical practice. Mallmann et al. [20] published the only pilot study in which, with the FISH technique, he detected bacteria in 26 out of the 54 heart valves and allowed a successful diagnosis of infective endocarditis in five out of 13 cases with negative blood cultures. Isolated studies conducted in patients with IE by Coxiella burnetii also recommend this technique to improve diagnostic yield [21-23]

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Mass spectrometry: MALDI-TOF

Mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), is a technique used in the identification of microorganisms by creating a spectrum based on the protein profile, which is unique to a given species. Numerous studies have shown in recent years the advantages of the use of MALDI-TOF for bacterial identification [24,25]. Several papers that analyze the profitability of this technique in blood cultures [26,27], but not specifically in IE have also been published. As a matter of fact, articles published by IE that use the MALDI TOF technique for diagnosis always refer to isolated cases in which an unusual pathogen is involved and difficult to diagnose with traditional culture techniques [28-30]. Although MALDI-TOF is undoubtedly a very promising technique that can contribute to substantially reduce the time of diagnosis in IE and as a consequence to a more adequate management, it is neither widely available nor is its use in these infections standardized at the moment. The application of all techniques alone or in combination will contribute in the immediate future to reducing the number of BCNE, increasing the diagnostic accuracy of the Duke criteria and will possibly have a positive impact on the morbidity and mortality associated with this life-threatening condition.

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