Clinical Perspective

Molecular Diagnosis of Infective Endocarditis: A Helpful Addition to the Duke Criteria

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Infective endocarditis (IE) remains a disease of concern because of its relatively high morbidity and mortality if not treated aggressively. Its incidence rate is between 1.7 to 6.2 cases per 100,000 in the general population and has remained essentially unchanged in the last two decades.¹ Presently, there is a shift in the demographics of populations at risk, with IE being seen more often in intravenous drug users,² patients exposed to nosocomial settings,³ and hemodialysis patients.⁴ A number of review articles on IE are available for a detailed discussion of this topic.^{1,5-8}

The current Duke classification criteria are used worldwide and are extremely useful in the diagnosis of native valve IE. These diagnostic guidelines include major and minor criteria, such as positive microbiological cultures, vegetations seen by echocardiography, fever, and the presence or absence of a predisposing heart condition. The Duke criteria have been found to be more sensitive than the von Reyn criteria in identifying IE cases as shown by Andrs et al.⁹ in a study of 38 patients in a clinical internal medicine practice. The enhanced sensitivity was due to the incorporation of echocardiographic findings in the Duke criteria. However, the utility of the Duke criteria has not been adequately assessed in patients with proven or suspected prosthetic valve endocarditis (PVE) or in selected populations, such as patients with congenital heart disease, in patients on chronic dialysis, or in hospitalized patients with indwelling catheters and bacteremia. In a study by Ben-Ami et al.,⁸ the rate of hospital-associated IE was 27 cases per 100,000 persons, suggesting a broadening of the definition of hospital-acquired IE.

Development of vegetation on the cardiac valves is a complicated process, typically starting with the adherence of microorganisms from transient bacteremia in susceptible valves. The damaged tissue provides a coagulum platform consisting of fibrin, fibronectin, plasma proteins, and platelet proteins for bacteria to adhere. This complex induces production of cytokines and procoagulant factors which in turn results in enlargement of the vegetation. The presence of fibronectin binding proteins on the coagulum enhances the attachment of some bacteria like *Staphylococcus aureus* to the substrate. In *S. aureus*, genes such as *agr* and *sar* can regulate the secretion of hemolysins and enterotoxins for further invasion.⁷

Currently, *S. aureus*, *Streptococcus* species, and enterococci account for more than 80% of the agents causing IE. Approximately 12% of the cases are due to culture-negative endocarditis which could be due to 1) fastidious organisms that defy culturing in routine microbiological media due to lack of appropriate growth factors,⁶ 2) ongoing chemotherapeutic treatment, and 3) very recent antibiotic

treatment. In a small number of cases, the agent can be grown in a microbiology laboratory, but cannot be identified at the species level using available biochemical tests. Some examples of common agents for culture negative endocarditis are *Coxiella burnetii*, species of *Bartonella*, *Brucella*, and *Mycoplasma*.

Still, a major criterion and method of choice in the diagnosis of endocarditis is isolation of an IE-associated microorganism from two to three separate blood cultures within 24 hours. If bacteremia is present, the first two blood cultures will yield the etiologic agent in more than 90% of the cases. Additional blood cultures may be needed if the patient received antibiotics in the preceding 2 weeks. In most cases the pathogen is identified by routine microbiological tests. However, if culture and biochemical tests fail to identify the organism, molecular methods, such as broad-range prokaryotic polymerase chain reaction (PCR) in conjunction with DNA sequencing of the amplicon and/or the use of specific probes, could be used. Pathogens are identified in the molecular method by amplifying and sequencing a target gene of the etiological agent that allows sufficient discrimination among different bacterial species. The 16S rRNA gene is still the most favored target gene for the identification of fastidious and uncultivable bacteria due to 1) enough discriminatory regions in this gene among different bacterial species, and 2) a large 16S rDNA sequence database available from GenBank (National Center for Biotechnology Information). This molecular approach could be used with a variety of clinical samples, such as blood or infected valve tissue, or the unidentified bacterial colony on solid medium.^{7,10} The DNA sequence of the target gene is then compared to the available sequences in GenBank. In almost every case, GenBank searches yield a list of bacterial names with a sequence identity index score for the query sequence. In general, a sequence identity of >97% of the nucleotides with the 16S rRNA gene from a known bacterium is considered a good match (provisional identification). Occasionally, the query sequence will yield more than one species with >97% sequence identity through the BLAST search feature of the National Center for Biotechnology Information. In such cases, but also in general, available phenotypic properties including results from the Gram stain should be taken into account before a final identification is made. High quality and nearly full-length consensus sequence data from both sense and antisense strands are prerequisites for a valid search in the sequence database and for reliable interpretation. Partial sequence data could lead to wrong identification in unusual cases.^{10,11}

Serology, with or without cell culture, has diagnostic value in identifying IE caused by *Coxiella burnetii*, and species of *Bartonella*, *Brucella*, *Chlamydia*, *Legionella*, and *Mycoplasma*.^{12,13} Bacteria that need to be grown in cell culture could be assayed with immunofluorescent antibodies for identification. Although rarely utilized in clinical practice, histological staining of biopsy material provides a direct observation of the inflammatory response and can be useful in detecting an etiological agent. A number of stains,

such as the Gram stain and periodic acid Schiff, each with its respective advantages and disadvantages, are routinely used.

An extremely fastidious organism, *Tropheryma whippelii* is very difficult to be isolated in culture¹⁴ and could be under-reported in IE cases if one strictly follows the Duke criteria. Although most IE associated microorganisms are detected by culture and occasionally by serology, diagnosis in the coming decade will likely rely more on the PCR-based methods because of their accuracy, efficiency, and expected widespread availability for identification.⁷ It has already been shown that the identification of *Bartonella* species, *C. burnetii*, and many other fastidious organisms is achieved more efficiently with the PCR assay.^{7,12,15}

In studies comparing blood culture and PCR-based methods, patients with definitive or possible IE were almost always found to be positive for a bacterium by PCR, even if their blood culture was negative.¹¹ The PCR-based method is almost indispensable in cases of endocarditis when ongoing antibiotic use may have rendered the blood sterile. Whenever feasible, the use of molecular based methods is generally recommended in IE cases where routine laboratory culture failed to identify the pathogen. Some authors have proposed to include the molecular method as a major criterion of the Duke classification system for the diagnosis of IE.¹¹ We support such a proposal.

It is important to realize that the PCR-based method has both advantages and limitations. The advantages lie in its high sensitivity, quick turnaround time, and accurate identification. Availability of the LightCycler[®] further cuts down the reaction run time compared to standard thermocyclers. Molecular-based identification is almost indispensable in cases such as culture-negative blood samples and surgically resected tissue. The disadvantages include potential contamination of clinical samples with bacteria from the environment or extraneous DNA during PCR reaction set-up or amplification steps. Failure to amplify the expected amplicon due to PCR inhibitors that may be present in clinical samples¹⁶ or in transport medium is still an area of concern in molecular diagnostics. In order to avoid false-positive PCR results, reactions should be set up in duplicates along with appropriate positive and negative controls. Whenever possible, positive PCR results should be considered along with additional clinical and laboratory data before the final diagnosis is made. Another limitation of the PCR method is that it cannot provide antibiotic susceptibility data or minimal inhibitory concentration, as is possible in conventional culture methods. It is conceivable in the future to have a gene chip that contains a battery of probes specific to not only hundreds of bacterial species, but also their virulence factor and antibiotic-resistant gene markers from a list of pathogens commonly or rarely associated with IE. This kind of system could be automated for a quick turnaround time and for cost effectiveness.

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