Heart Valve Culture and Sequencing to Identify the Infective Endocarditis Pathogen in Surgically Treated Patients

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Background. **Testing excised valves in surgically treated infective endocarditis (IE) patients provides an opportunity to identify the microbial etiology of IE. Microbial sequencing (universal bacterial, mycobacterial, or fungal polymerase chain reaction followed by DNA sequencing) of valves can identify microorganisms accurately, but the value it adds beyond information provided by blood and valve cultures has not been adequately explored.**

Methods. **Three hundred fifty-six patients who underwent surgery for active IE from January 1, 2010, to January 1, 2013, were identified from our cardiovascular information registry and outpatient parenteral antibiotic therapy registry. Their records were reviewed to identify 174 patients whose valves were sent for sequencing. The microbial etiology of IE was defined using comprehensive clinical, pathologic, and microbiological criteria. Blood culture, valve culture, and valve sequencing were examined to determine how frequently they identified the definitive cause of IE.**

Results. **Of the 174 patients, 162 (93%) had acute inflammation on histopathologic examination of their valves. Valve sequencing was significantly more sensitive than valve culture in identifying the causative pathogen (90% versus 31%, $p < 0.001$), and yielded fewer false positive results (3% versus 33%, $p < 0.001$). The pathogen would not have been identified in 25 patients (15%) had it not been for valve sequencing. All the value provided by sequencing was attributable to bacterial DNA sequencing; mycobacterial and fungal sequencing provided no additional information beyond that provided by blood culture, histopathology, and valve culture.**

Conclusions. **Valve sequencing, not valve culture, should be considered the primary test for identifying bacteria in excised cardiac valves.**

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Identifying the specific microbial cause of infective endocarditis (IE) is central to selecting appropriate antimicrobial therapy. The presence of comorbid conditions, and the often complicated and prolonged illnesses among patients with IE, necessitates careful consideration of culture results before assigning causative status to microorganisms isolated in various cultures. Infection with fastidious microorganisms or preceding antibiotic exposure can result in a presentation of culture negative IE [1, 2]. Microbial nucleic acid sequencing has been shown to be useful in identifying fastidious microorganisms [3], and can also identify cultivable pathogens [4]. The purpose of this study was to examine the contribution of the three test modalities—blood culture, valve culture, and valve sequencing—in identifying the pathogen in patients who undergo surgery for IE.

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Patients and Methods

Setting

Explanted cardiac valves from patients with suspected IE in our institution are split for microbiological and pathologic examination in the operating room by the cardiac surgeons. Cardiac pathologists and infectious disease clinicians hold weekly valve pathology rounds to review cases of suspected IE. Since 2009, fresh cardiac valve specimens from many patients are sent for molecular testing when requested by the treating physicians [5]. The clinical specimens are sent to University of Washington Laboratories, Seattle, Washington, for molecular testing. Molecular testing involves amplification by polymerase chain reaction (PCR), followed by DNA sequencing of any amplified product. PCR includes one or more of the following: universal bacterial PCR, universal fungal PCR, or mycobacterial PCR. Universal bacterial PCR is done using broad-range 16S rRNA primers [5–7]. Universal fungal PCR is done using broad-range 28S rRNA and internal transcribed spacer 1 and 2 regions (ITS1 and ITS2) primers [8]. Mycobacterial PCR is done using broad-range 16S rRNA (same test as for universal bacterial PCR), and

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rpoB gene PCR. Sequencing of the amplified product yields the identification. Mycobacterial PCR additionally includes PCR of the hsp65 gene followed by hybridization probe identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare* [9]. All these tests are predicated on making an identification based on the nucleic acid sequence of the microorganism. In this paper, these molecular tests are collectively referred to as "sequencing" for the sake of brevity.

Patients

Patients undergoing surgery for IE from January 1, 2010, to January 1, 2013, were identified. Electronic records of all these patients were reviewed. Only patients with definite endocarditis by Duke criteria [10] whose excised cardiac specimens were sent for valve sequencing were included. Patients who did not have histologic evidence of endocarditis (acute inflammation or microorganisms on special staining) were excluded if they did not have the same pathogen identified by two of the three test modalities (blood culture, valve culture, and valve sequencing) used for microbial identification. These exclusions ensured that only patients with active IE were included, and also avoided the possibility that in any patient the Duke criteria were fulfilled only because of a microbiologic test modality that was being evaluated in this study.

Of 356 patients who underwent surgery during the defined study period and met Duke criteria for definite endocarditis, 177 patients had one or more specimens sent for sequencing. Three of these patients were excluded because they would not have met Duke criteria for definite endocarditis had it not been for identification of a microorganism by a single microbiologic test modality. That yielded 174 unique patients, and every patient was included only once.

Data

Three test modalities were evaluated: blood culture, culture of specimens obtained from the operative site (henceforth collectively referred to as valve culture), and microbial sequencing of specimens obtained from the operative site (henceforth collectively referred to as valve sequencing). Valve culture always included aerobic, anaerobic, mycobacterial, and fungal cultures. Valve sequencing always included bacterial sequencing, and sometimes fungal or mycobacterial sequencing. Results of the three test modalities were examined.

Analysis

For the purpose of the analysis, the definitive microbial cause of endocarditis was defined by following the criteria outlined in Table 1. The sensitivity of a test modality was defined as the number of patients whose pathogen was identified by the modality among all patients whose causative pathogen was known. Identification of a contaminant (microorganism that was not the cause of IE) was considered a false positive. For both these measures, the denominator excluded patients who were not tested by the modality in question. The sensitivities and false positive rates of the three test modalities were calculated. For the purpose of the analysis, a test modality was given full credit even if it identified a different species of coagulase negative staphylococcus or viridans group streptococcus than the definitive pathogen. If a modality had a false positive but also identified the definitive pathogen, the modality was not penalized for the false positive. In polymicrobial infections, a modality was given full credit if it correctly identified at least one of the pathogens causing endocarditis.

Results

Patient Characteristics

Of the 174 included patients, 46% (80 of 174) had native valve and 54% (94 of 174) had prosthetic valve endocarditis. Valve histopathologic examination was not performed in 8 patients. Of the 166 patients whose valves were examined

Table 1. Criteria Used to Define Cause of Endocarditis

The following would be considered causal for endocarditis:
Same pathogen in blood culture, valve culture, and valve sequencing.
Same pathogen in two of the three—blood culture, valve culture, and valve sequencing—with a clinical presentation consistent with the pathogen identified.
A pathogen identified by any one modality—blood culture, valve culture, or valve sequencing—would only be considered if the same pathogen is identified in more than one specimen, and if the clinical, operative, and histopathologic findings are consistent with endocarditis caused by that microorganism.
A pathogen identified by any one modality—blood culture, valve culture, or valve sequencing—in only one specimen would only be considered if endocarditis caused by the pathogen has been well described, and if the clinical, operative, and histopathologic findings are consistent with endocarditis caused by that microorganism.
The following would be excluded as the cause of endocarditis:
Single colony or rare growth of coagulase-negative staphylococci (CoNS), viridans streptococci, *Propionibacterium acnes*, corynebacteria, or *Bacillus* sp on a single valve culture, unless the same pathogen is also identified by a different modality.
Growth of CoNS, viridans streptococci, *P acnes*, corynebacteria, or *Bacillus* sp on a single valve culture, if there is another identified pathogen that could explain the endocarditis.
Positive sequencing for CoNS, viridans streptococci, *P acnes*, corynebacteria, or *Bacillus* sp on a single specimen, if there is another identified pathogen that could explain the endocarditis.
Growth of any pathogen that is clearly inconsistent with endocarditis when interpreted in the context of clinical presentation, operative findings, histopathologic findings, and other microbiological findings.