Contribution of Systematic Serological Testing in Diagnosis of Infective Endocarditis

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Despite progress with diagnostic criteria, the type and timing of laboratory tests used to diagnose infective endocarditis (IE) have not been standardized. This is especially true with serological testing. Patients with suspected IE were evaluated by a standard diagnostic protocol. This protocol mandated an evaluation of the patients according to the modified Duke criteria and used a battery of laboratory investigations, including three sets of blood cultures and systematic serological testing for Coxiella burnetii, Bartonella spp., Aspergillus spp., Legionella pneumophila, and rheumatoid factor. In addition, cardiac valvular materials obtained at surgery were subjected to a comprehensive diagnostic evaluation, including PCR aimed at documenting the presence of fastidious organisms. The study included 1,998 suspected cases of IE seen over a 9-year period from April 1994 to December 2004 in Marseilles, France. They were evaluated prospectively. A total of 427 (21.4%) patients were diagnosed as having definite endocarditis. Possible endocarditis was diagnosed in 261 (13%) cases. The etiologic diagnosis was established in 397 (93%) cases by blood cultures, serological tests, and examination of the materials obtained from cardiac valves, respectively, in 348 (81.5%), 34 (8%), and 15 (3.5%) cases. The etiologic diagnosis was established in definite cases of IE. Concomitant infection with streptococci and C. burnetii was seen in two cases. The results of serological and rheumatoid factor evaluation reclassified 38 (8.9%) possible cases of IE as definite cases. Systematic serological testing improved the performance of the modified Duke criteria and was instrumental in establishing the etiologic diagnosis in 8% (34/427) cases of IE.

In recent decades, standard diagnostic schemes have been developed to improve the sensitivity and specificity of the diagnosis of infective endocarditis (IE) (12, 13). The von Reyn criteria (37), introduced in the early 1980s, have been abandoned in favor of the modified Duke criteria (18, 19, 29). The introduction of transeosophageal echocardiography into clinical practice is an important step towards improved sensitivity and specificity in the diagnosis of infective endocarditis. The incorporation of diagnostic imaging features of IE, obtainable by echocardiography, in the Duke criteria has considerably improved the diagnosis sensitivity of these criteria.

Despite advances in diagnostic techniques, etiologic diagnoses cannot be obtained in 2.5 to 31% cases of IE (1, 3, 6, 22, 24, 29, 30, 36). These so-called blood culture-negative cases of endocarditis (BCNE) often pose considerable diagnostic and therapeutic dilemmas. First, BCNE are often caused by obligate intracellular bacteria, fungi, and fastidious organisms (1). Isolation of these organisms requires culturing them on specialized media, and their growth is relatively slow on artificial culture media. Second, the institution of appropriate antibiotic treatment is often delayed in cases where endocarditis is caused by one of the organisms indicated above and may adversely affect the outcome of treatment (8). Finally, the modified Duke criteria perform poorly in patients with BCNE (24), leaving room for further modification of these criteria to improve their diagnostic performance in cases of BCNE.

Unlike the role of microbiological cultures, the role of serological testing in the etiological diagnosis of IE is not completely established, despite the exception represented by Coxiella burnetii. We are not aware of any report published in the English language literature that has systematically evaluated the role of serological testing in establishing the etiological diagnosis of IE. This is unfortunate, considering the logistic problems enumerated above that impose restrictions on the application of microbiological culture methods for the etiological diagnosis of IE in every setting. Furthermore, a better definition of the role of serological testing in the diagnosis of IE may increase the number of patients who are labeled “definite cases” by the modified Duke criteria. Of note, a positive serological test for Coxiella burnetii has already been included as a major criterion in the modified Duke criteria (13).

Therefore, we sought to assess the contribution of systematic serological testing in arriving at the correct etiologic diagnosis of IE. More specifically, we examined whether systematic serological testing would have any effect in decreasing the frequency of a diagnosis of endocarditis without a microbiologic diagnosis, as well as the interval between clinical suspicion and initiation of specific antimicrobial therapy in patients suspected of having IE.

MATERIALS AND METHODS

All patients admitted into L’Hôpital de la Timone, University of Marseilles, France, between April 1994 and December 2004 with clinical suspicion of IE were enrolled in a prospective study. The study protocol was approved by the
The modified Duke criteria (Table 1) were used to define cases of endocarditis. Sixty-six percent (1,310/1,998) were rejected. Definite cases. Of the 427 definite cases, 318 (74.5%) had predisposing factors including valvular heart disease in 179 (41.9%), biological heart prostheses in 68 (16%), mechanical prostheses in 41 (9.6%), cardiac pacemakers in 64 (15%), and ventriculoatrial shunt in 1.

The etiologic diagnosis was established in 397 (93%) of 427 definite cases. This was possible (Table 3) by blood culture, serological testing, PCR analyses of samples obtained from cardiac valves, and culturing materials obtained from cardiac valves in 348 (81.5%), 34 (8%), 11 (2.6%), and 4 (0.9%) cases, respectively.

Of the 348 organisms isolated from blood cultures (Table 4), 239 (68.7%) fulfilled major and 109 (31.3%) fulfilled minor Duke criteria. The results of serological and rheumatoid factor testing made it possible to reclassify 38 possible cases as definite cases of IE, which represented 8.9% of the total cases of definite endocarditis. This reclassification was the result of positive serological tests for C. burnetii in 22 cases, Bartonella spp. in 5 cases, Legionella spp. in 2 cases, and Aspergillus spp. in 1 case later confirmed by valve culture. Of importance, 22 patients had antibodies to Bartonella spp., but only the 5 patients with high titers (≥400) had evidence of IE (Table 4).

Serological testing also identified two patients with dual infection. One had concomitant infection with Coxiella burnetii (confirmed by both serology and culture) and Streptococcus bovis. The other had concomitant infection with Coxiella burnetii and Streptococcus mitis, confirmed by PCR performed on a valve specimen. The serological testing for rheumatoid factor contributed to the diagnosis of eight cases of definite endocarditis.

As previously mentioned, blood cultures yielded a microorganism in 348 of 427 definite cases of IE (81.5%), including 62 patients who had received prior antimicrobial therapy.

TABLE 1. Proposed modified Duke criteria for definition of IE

<table>
<thead>
<tr>
<th>Criterion category</th>
<th>Proposed modified Duke criterion</th>
<th>a</th>
</tr>
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<tbody>
<tr>
<td>Major ..................Blood culture typical of IE, single positive culture for C. burnetii, or immunoglobulin G antiphase I level of ≥1/800</td>
<td></td>
<td></td>
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<tr>
<td>Echocardiogram positive for IE</td>
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<td></td>
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<tr>
<td>New valvular regurgitation</td>
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<td></td>
</tr>
<tr>
<td>Minor ...............Predisposition (predisposing heart condition or injection drug use)</td>
<td></td>
<td></td>
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<tr>
<td>Fever (temperature of &gt;38°C)</td>
<td></td>
<td></td>
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<tr>
<td>Vascular phenomena</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunologic phenomena (including rheumatoid factor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiologic evidence, positive blood culture (not meeting major criterion), or serological evidence of active infection</td>
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</table>

a Items specifically tested in this study are shown in boldface type. See Table 2 for definitions of IE.

TABLE 2. Definition of IE according to the proposed modified Duke criteria

<table>
<thead>
<tr>
<th>Category of IE</th>
<th>No. and type(s) of criteria that must be met for diagnosis or reason(s) for rejection of diagnosis</th>
</tr>
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<tbody>
<tr>
<td>Definite ..........(i) 2 major criteria, (ii) 1 major criterion and 3 minor criteria, or (iii) 3 minor criteria</td>
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<tr>
<td>Possible ..........(i) 1 major criterion and 1 minor criterion or (ii) 3 minor criteria</td>
<td></td>
</tr>
<tr>
<td>Rejected ..........(i) Firm alternative diagnosis explaining evidence of IE; (ii) resolution of IE syndrome with antibiotic therapy for ≥4 days; (iii) no pathologic evidence of IE at surgery or autopsy, with antibiotic therapy for ≥4 days; or (iv) does not meet criteria for possible IE</td>
<td></td>
</tr>
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</table>

a See Table 1 for modified Duke criteria.
In 15 cases with both negative blood cultures and serological testing, an etiologic diagnosis was determined by analysis of the valve after surgery (Table 4). All these patients had received antimicrobials before blood cultures were obtained; as a result, the patients were considered to have BCNE (9). Two valves had cultures that were positive for fungi (Acremonium spp. and Aspergillus spp.), 2 had cultures positive for bacteria (Escherichia coli and Propionibacterium acnes), and 11 were positive by PCR testing (Table 3).

Based on Duke criteria, a diagnosis of definite endocarditis without etiology was made in 30 patients (7%) who were pathologically proven but had a negative broad-spectrum PCR of the valve.

Possible cases. Among the 261 possible cases, 16 (6%) had either positive blood cultures (n = 12) with organisms fulfilling major microbiological criteria, as well as predisposing heart conditions, or a positive serological test (n = 4) but had inconclusive transesophageal echocardiography. The results of serological and rheumatoid factors testing made it possible to reclassify 19 rejected cases as possible cases of IE. Forty-one (16%) patients were considered to have IE and were treated accordingly. At a 6-month follow-up, none of the 220 untreated patients with possible IE exhibited evidence of ongoing IE.

Rejected cases. Of the 1,310 rejected cases, 11 (0.8%) had positive blood cultures fulfilling major Duke microbiological criteria. Thirty-six (2.7%) had positive serological tests. However, a careful evaluation failed to discover any evidence of endocarditis in these patients.

Rheumatoid factor. The latex agglutination test for rheumatoid factor was positive in 164 (8%) cases. The test was significantly more often positive (P = 0.006) in definite cases (48/427) than in possible cases (20/261), rejected cases (96/1,310), or asymptomatic blood donors (7/200).

Histological evaluation of cardiac valves. A total of 292 patients underwent cardiac valve replacement. Histological examination of the valve materials confirmed the diagnosis of IE in 142 definite cases of endocarditis. PCR and microbiological culture established the etiologic diagnosis in 11 and 4 cases, respectively (Table 3).
DISCUSSION

The objective was to evaluate the role of systematic serological testing in the diagnosis of IE. Our data do, in fact, show that careful and systematic serological evaluation plays an important role in the diagnosis of IE. This is supported by the fact that a systematic serological evaluation, along with other diagnostic modalities, allowed us to make a diagnosis of definite endocarditis in 21% (427 of 1,998) of suspected cases of infected endocarditis seen at L’Hôpital de la Timone between April 1994 and December 2004. A diagnosis of possible endocarditis was obtained in another 13% (261 of 1,998) of cases.

More importantly, results of the systematic serological testing reclassified 34 possible cases of endocarditis as definite cases of IE and 2 rejected cases as possible cases of endocarditis. These data suggest that systematic serological evaluation may add to the discriminatory power of the Duke criteria. This is understandable, considering the fact we have stratified our cases of suspected endocarditis into different diagnostic classes according to the modified Duke criteria (Table 1). In our study, even systematic study of rheumatoid factor was found to be useful, as it enabled us to reclassify eight possible cases of endocarditis as definite cases (13).

BCNE remains an Achilles heel in the diagnosis and treatment of IE. Despite advances in microbiological culture and molecular and immunohistochemical techniques, 2.5 to 31% of all cases of endocarditis remain without a microbiological diagnosis (6). Various factors have been linked to the existence of culture-negative IE. These include, among others, difficulties in isolating fastidious organisms that are often the causative agents of culture-negative endocarditis (7, 9, 15, 20, 39) and the institution of antibiotic treatment before cultures are obtained. While not deemphasizing the roles of diagnostic tests, we believe that culture-negative endocarditis is also, to a great extent, a problem of the lack of application of these tests. A systematic approach with the judicious use of serological tests and, where indicated, the use of advanced molecular diagnostic methods and immunohistochemical techniques may elucidate etiologic diagnosis for many patients with BCNE (17, 29, 31, 39). The prevalence of zoonotic agents causing endocarditis, such as C. burnetii, Brucella spp., and Bartonella spp., may vary widely in different geographic areas. Now, we know that 15% of cases of endocarditis in Algeria (4) and 10% of cases of endocarditis in Tunisia (42) are caused by Cxoxiella burnetii and Bartonella spp. The prevalence of Bartonella spp. in this context is very low in Sweden (38) and reaches 3% in France (32), maritime Canada, and Germany (14).

Study design precluded us from analyzing the cost effectiveness of our strategy. However, our results suggest that diagnostic strategy we used in Marseilles would be cost effective. This is understandable, since such a strategy would recommend a battery of diagnostic tests (e.g., blood cultures, serological testing, and echocardiography) immediately after a clinical diagnosis of endocarditis is made. Furthermore, the results of such diagnostic tests would be obtained within 5 days following admission. Obviously, it would likely shorten the delay before specific treatment was instituted. Contrary to common practice, clinicians in such a situation would not have to defer serological testing until the blood cultures were shown to be negative. Instead, clinicians could perform both blood cultures and serological tests at the same time. We strongly feel that the savings that made by shortening the length of hospitalization by a single day would suffice to make up the extra costs of the battery of serological tests we have incorporated, as above, into our diagnostic strategy. In our study, an etiologic diagnosis was obtained within 5 days for 94% of all patients with definite IE.

Conclusions. Initial serological testing on admission is useful in stratifying patients with suspected IE as “definite,” “possible,” or “rejected” cases of IE. This testing is also useful in establishing the etiologic diagnosis of IE.

ACKNOWLEDGMENTS

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REFERENCES