

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 Marseille, 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%) 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 transesophageal 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 Marseille, 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

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TABLE 1. Proposed modified Duke criteria for definition of IE

Criterion category	Proposed modified Duke criterion ^a
Major	Blood culture typical of IE, single positive culture for <i>C. burnetii</i>, or immunoglobulin G antiphase I level of $\geq 1/800$ Echocardiogram positive for IE New valvular regurgitation
Minor	Predisposition (predisposing heart condition or injection drug use) Fever (temperature of $>38^{\circ}\text{C}$) Vascular phenomena Immunologic phenomena (including rheumatoid factor) Microbiologic evidence, positive blood culture (not meeting major criterion), or serological evidence of active infection

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

institutional Review Board and Ethics Committee of the University of Marseilles. Informed consent was obtained from all study subjects before enrollment in the study.

A structured questionnaire was used to collect the following data: patient's age, sex, signs and symptoms, duration of symptoms, history of antimicrobial therapy for the current illness that prompted the patient to seek medical attention, antecedent disease, predisposing factors for IE (including systemic disease, prosthetic valve, intravenous drug abuse, and dental or surgical manipulation), treatment received during the course of hospitalization, and outcome of treatment.

Each patient underwent testing that was guided by a diagnostic kit. Each kit contained written guidelines for testing requirements and an informed consent form. The kit was composed of three units. The first, to be used immediately, included a set of two blood culture vials for aerobic and anaerobic cultures (BACTEC; Becton Dickinson, Sparks, MD) and a tube to collect a serum sample used for rheumatoid factor detection (Rapitex RF; Dade Behring, Inc., Newark, NJ) (33) and estimation of specific antibodies directed against *C. burnetii* (34), *Bartonella* spp. (14), *Brucella* spp., *Chlamydia* spp. (5), *Mycoplasma pneumoniae* (2), *Legionella pneumophila* (40), and *Aspergillus* spp. (35). As previously demonstrated in our laboratory (21, 28), a cutoff titer for *Bartonella* spp. of 400 was used to diagnose endocarditis. Similarly, *C. burnetii* antiphase I immunoglobulin G titers of >800 were considered a major criterion for the diagnosis of IE. The second and third units of the diagnosis kit each contained a set of two blood culture vials to use 2 and 4 h, respectively, after the first one (10).

Bacterial identification was performed according to a clinical microbiology procedures handbook (23). When usual methods were inconclusive, PCR amplification, followed by sequencing of the 16 rRNA gene, was performed (11, 15, 16). DNA extracts were prepared from suspect colonies for use as templates in PCR amplification with the QIAmp Blood kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions.

Valvular surgical samples included valvular and periprosthetic tissue, vegetations, valve prostheses, and tissue fragments obtained from abscess debridement. A smear of each sample was prepared and stained using Gram and Ziehl-Neelsen methods. As described above for bacterial identification, a part of each tissue sample was inoculated into brain heart medium and blood agar medium, as well as into a cell culture. A DNA extract, suitable for use as a template for PCR as described above, was prepared from each sample with the QIAmp tissue kit (QIAGEN), according to the manufacturer's instructions (17). For pathological examinations, formalin-fixed and paraffin-embedded tissue samples were cut to a 5- μm thickness and stained with routine hematoxylin-eosin stain. Valvular tissue examination enabled the recognition of consistent patterns of tissue damage associated with IE, namely, vegetation and valvular inflammation (25). Special stains were used to detect bacteria and fungi: Giemsa, Brown-Brenn, and Brown-Hopps tissue Gram, periodic acid-Schiff, Gimenez (7), Grocott-Gomori methenamine silver, Ziehl-Neelsen, and Warthin-Starry (26, 41).

The modified Duke criteria (Table 1) were used to define cases of endocarditis (Table 2) (27). All patients assessed as having possible IE received antibiotic treatment if they had one major Duke criterion, echocardiographic abnormalities, or a microbiologically proven infection. All cases of possible IE were followed up for a minimum period of 6 months.

Data were expressed as means \pm standard deviation or number and percent-

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

Category of IE	No. and type(s) of criteria that must be met for diagnosis or reason(s) for rejection of diagnosis
Definite.....	(i) 2 major criteria, (ii) 1 major criterion and 3 minor criteria, or (iii) 5 minor criteria
Possible.....	(i) 1 major criterion and 1 minor criterion or (ii) 3 minor criteria
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

^a See Table 1 for modified Duke criteria.

age of patients. Differences in categorical variables were tested by the chi-square test. Statistical significance was defined as *P* values of <0.05 .

RESULTS

During the study period, a total of 1,998 patients were admitted at L'Hôpital de la Timone with clinical suspicion of endocarditis. Twenty-one percent (427/1,998) had definite endocarditis, and thirteen percent (261/1,998) had possible endocarditis, as categorized by the Duke criteria. 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 infections. 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 3. Definite IE final diagnosis obtained with IE diagnostic kit: microbiological data

Method of detection and causative organism	No. of cases (%)
Blood culture.....	348 (81.5)
<i>Staphylococcus aureus</i>	78
<i>Streptococcus bovis</i>	67
Viridans streptococci.....	50
Coagulase-negative staphylococcus.....	50
<i>Enterococcus faecalis</i>	28
<i>Escherichia coli</i>	10
Other enterococci.....	8
<i>Enterococcus durans</i>	3
<i>Enterococcus faecium</i>	2
<i>Enterococcus</i> spp.....	2
<i>Enterococcus avium</i>	1
HACEK group.....	8
<i>Actinobacillus actinomycetemcomitans</i>	5
<i>Haemophilus aphrophilus</i>	1
<i>Haemophilus parainfluenzae</i>	1
<i>Cardiobacterium hominis</i>	1
<i>Streptococcus agalactiae</i>	6
<i>Candida</i> spp.....	6
<i>Streptococcus pneumoniae</i>	5
<i>Actinobacillus</i> spp.....	5
<i>Gemella</i> spp., group G streptococcus, <i>Enterobacter cloacae</i> , and <i>Corynebacterium</i> spp.....	3 each
<i>Acinetobacter</i> spp., <i>Abiotrophia defectiva</i> , and <i>Campylobacter fetus</i>	2 each
<i>Neisseria sicca</i> , <i>Ralstonia pickettii</i> , <i>Pseudomonas aeruginosa</i> , <i>Chryseomonas</i> , <i>Klebsiella pneumoniae</i> , <i>Proteus mirabilis</i> , <i>Peptostreptococcus</i> spp., <i>Propionibacterium acnes</i> , and <i>Listeria monocytogenes</i>	1 each
Serology.....	34 (8)
<i>Coxiella burnetii</i>	26
<i>Bartonella</i> spp.....	5
<i>Legionella pneumophila</i>	2
<i>Aspergillus</i> spp.....	1
Valve analysis.....	15 (3.5) ^a
Culture	
<i>Aspergillus</i> spp., <i>Acremonium</i> spp., <i>Escherichia coli</i> , and <i>Propionibacterium acnes</i>	1 each
PCR	
Viridans streptococci.....	3
<i>Streptococcus bovis</i>	2
<i>Granulicatella elegans</i> , <i>Mycoplasma hominis</i> , <i>Streptococcus pneumoniae</i> , <i>Streptococcus anginosus</i> , <i>Streptococcus agalactiae</i> , and <i>Cardiobacterium hominis</i>	1 each
No etiology found.....	30 (7)
Total no. of IE cases.....	427

^a The number of cases where the organism was detected by culture was 4 (0.9%); the number of cases detected by PCR was 11 (2.6%).

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

TABLE 4. Results of biological tests for suspected cases of IE, according to modified Duke criteria

Positive test	Total (1,998)	Duke classification (no. of cases)		
		Definitive IE (427) ^a	Possible IE (261) ^a	Rejected IE (1,310)
Blood culture	432	348	20	64
Major criterion	262	239	12	11
Minor criterion	170	109	8	53
Serology	57	34 (30)	2 (2)	21
<i>C. burnetii</i> major	33	26 (22)	1 (1)	6
<i>Bartonella</i> (titers of ≥ 400)	5	5 (5)	0	0
<i>Legionella</i>	11	2 (2)	0	9
<i>Aspergillus</i>	1	1 (1)	0	0
<i>Chlamydia</i>	2	0	1 (1)	1
<i>Mycoplasma pneumoniae</i>	4	0	0	4
<i>Brucella</i>	1	0	0	1
Rheumatoid factor	164	48 (8)	20 (17)	96
Total no. of upgraded cases		38	19	

^a Values in parentheses are numbers of cases upgraded in the Duke criteria by test results.

(*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%): 11 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 cases 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 *Coxiella 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 serologi-

cal 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.

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Potential conflicts of interest: D.R. has a patent pending on serological diagnosis of endocarditis.

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