PCR Detection of Bacteria on Cardiac Valves of Patients with Treated Bacterial Endocarditis

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We used broad-range PCR amplification and sequencing to detect and identify bacterial DNA in 156 valves of patients treated for infective endocarditis (IE). Bacterial DNA was found more frequently in patients who underwent valve replacement while on antibiotic treatment for IE (60%) than in patients who had completed antibiotic treatment for IE (37%; $P = 0.02$). We found specific bacterial DNA in valves removed from 11 of 30 patients who had completed antibiotic treatment for IE. Six had no histological evidence of IE. The presence of DNA was significantly correlated with the presence of histologic lesions ($P = 0.001$) and with the presence of bacteria detected by Gram staining ($P < 0.001$). *Bartonella* and streptococci were detected for much longer after antibiotic treatment by PCR than other species ($P = 0.047$ and 0.04, respectively), and coagulase-negative staphylococci were detected for much shorter periods ($P = 0.02$). The finding that bacterial DNA was more likely to be detected in valves of patients with active IE than in patients who had completed antibiotic treatment for IE shows that bacterial DNA is cleared slowly. There was no significant correlation between the duration of antibiotic therapy and the presence of bacterial DNA in valves. Since the persistence of bacterial DNA in valves does not necessarily indicate the persistence of viable bacteria, the detection of bacterial DNA in valves from IE patients should be interpreted with caution, in particular in those patients with a past history of treated IE.

Direct amplification of bacterial DNA from normally sterile sites by using broad-range primers provides an alternative approach to the detection of pathogens. Amplification and sequencing of the 16S rRNA gene in DNA extracted from cardiac-valve tissue has been used to provide etiological diagnoses in patients with culture-negative endocarditis (8, 11, 13, 15, 30, 35, 47). Consequently, Bosshard et al. and Millar et al. proposed that the Duke criteria should be modified to include a molecularly based diagnosis of noncultured causal agents as an additional major criterion (4, 28).

One problem is that bacterial DNA can still be detected after bacterial cultures become sterile. In patients with septic arthritis, bacterial DNA can be found in synovial tissue for long periods after cultures become negative (44). The persistence of bacterial DNA despite effective antibiotic treatment has also been demonstrated in patients with pulmonary tuberculosis (14, 49) and leptospirosis (3, 27). DNA is sufficiently stable that it can be amplified by PCR for long periods after bacteria are no longer viable (7, 25). As examples, PCR can be used to detect DNA in cells from mummies (31), *Yersinia pestis* DNA was found in 500-year-old human dental pulp (37), and mycobacterial DNA was detected in ancient human corpses that had macroscopic signs of tuberculosis (43) and leprosy (36). In a recent study, the presence of histological signs of infective endocarditis (IE) correlated with the presence of bacterial DNA in heart valve tissue, and when patients had no histolog-

ical evidence of IE, PCR amplification was always negative (10). A recent study has shown that the presence of Gram-staining organisms in heart valves of patients cured of IE does not predict the presence of viable organisms (29). However, it has not been determined whether DNA amplification from valve tissue is predictive of an active infectious process. If this were the case, it might represent a tool to assess the efficiency of antibiotic therapy.

Since 1994, we have routinely used broad-range PCR amplifying the 16S rRNA-encoding gene and sequencing to detect and identify bacterial DNA in heart valve samples. In this report, our goal was to evaluate the role of antibiotic treatment duration in the persistence of bacterial DNA during endocarditis. We describe the experiments we performed to determine whether amplification of bacterial DNA in valve tissue was correlated with the duration of antibiotic treatment in patients with IE.

MATERIALS AND METHODS

Patients. In a prospective study, we enrolled patients with endocarditis diagnosed according to the modified Duke's criteria described by Li et al. (23). There was a known etiology for their IE (by culture or serology), and the patients had been or were being treated with antibiotics when they had valve replacement surgery between 1 April 1994 and 31 April 2003. Infections with *Bartonella* spp. were diagnosed by culture or amplification of *Bartonella* DNA from valve tissue or whole blood or by serology with immunoglobulin G titers of ≥1:800 by microimmunofluorescence (38). For IE due to viridans streptococci or enterococci, effective treatment was defined using the recommendations of Wilson et al. (48), and for IE due to staphylococci, effective treatment was defined using the recommendations of Rubinstein and Carbon (40). For *Bartonella* spp., the recommendations of Raoult et al. were used to define effective treatment (38). For other organisms, antibiotic treatment was regarded as effective if antibiotics were administered intravenously according to the results of antimicrobial sensitivity

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TABLE 1. Characteristics of 156 episodes of valve replacement for IE according to PCR

Parameter	Value for group		P value
	Positive PCR (n = 87)	Negative PCR (n = 69)	
Demographic data			
No. (%) male	62 (71)	49 (70)	0.82
Median (IQR) age (yr)	58 (42–70)	60 (50–69)	0.27
Histologic data			
Histologic lesions in favor of IE[a] [no. (%)]	64 (75)	31 (48)	0.001
Presence of bacteria on Gram staining[b] [no. (%)]	43 (54)	15 (24)	<0.001
Etiologic diagnosis [no. (%)]			
Streptococcus spp.	46 (53)	28 (41)	0.13
S. bovis	22 (25)	12 (17)	0.24
Oral Streptococcus	19 (22)	15 (22)	0.99
Enterococcus spp.	10 (11)	8 (12)	0.99
Enterococcus spp. and Streptococcus spp.	56 (64)	36 (51)	0.12
Staphylococcus spp.	18 (21)	23 (33)	0.08
CNS	4 (5)	9 (13)	0.06
S. aureus	14 (16)	14 (20)	0.50
Bartonella spp.	7 (8)	0 (0)	0.02
Others[c]	11 (13)	11 (16)	0.56

[a] Six missing valve for histologic study.
[b] Fourteen missing valve for Gram-staining study.
[c] Others: Actinobaterium actinomycetans (three cases), Corynebacterium jeikeium, Corynebacterium diphteriae, Proteus mirabilis, Haemophilus spp. (two cases), Streptococcus pneumoniae (two cases), Proprionobacterium acnes, Micrococcus spp., Enterobacter cloacae (two cases), E. coli (two cases), Streptococcus β haemolytic (three cases), Streptococcus agalactiae, Neisseria sicca, rastonia.

testing. The days of prescription of parenteral antimicrobial treatment with more than one active antibiotics were counted. Oral agents taken after completion of effective treatment were included in the duration of treatment. Patients who received antibiotics only orally and those receiving insufficient doses of antibiotics were considered to have had insufficient treatment. Patients with a history of IE but for whom the type and duration of antibiotic therapy were not known were considered to have been treated effectively if they had no signs of active endocarditis at the time of surgery. Patients with IE due to multiple bacteria or with fungal endocarditis were excluded from the study. Patients with Coxiella burnetii endocarditis were excluded because it is a chronic condition that responds poorly to therapy (22) and would have biased the study. Patients were also excluded if the sequencing results indicated an etiological agent different from that obtained by culture or serology. Our selection criteria enabled us to group patients into those who, at the time of valve replacement surgery, had active IE and were receiving antibiotic treatment and those who had completed antibiotic treatment at the time of surgery.

Microbiology. Three aerobic (BACTEC PLUS Aerobic/F) and anaerobic (BACTEC LYTIC/10 Anaerobic/F) blood cultures were obtained from each patient and incubated for 1 week in the BACTEC 9240 blood culture system (Becton Dickinson, Sparks, Md.). Valve tissue from patients was inoculated onto 5% blood agar (bioMérieux, Marcy-l'Etoile, France) and chocolate agar (bioMérieux) and incubated at 37°C in a 5% CO_2 atmosphere for 10 days. Valve samples were also cultured on BCYE (bioMérieux) for 15 days and on Columbia media, where they were incubated under anaerobic conditions for 10 days. To isolate Bartonella spp., whole blood and valve tissue were inoculated onto both 5% blood agar (bioMérieux) and ECV 34 (20) and incubated at 37°C in a 5% CO_2 atmosphere for up to 60 days. Isolates obtained from cell culture were identified by using species-specific mouse polyvalent antisera (26) and PCR (16, 39).

PCR amplification and sequencing. Heart valve samples were stored at −80°C prior to DNA extraction, which was performed with the QIAmp kit (QIAGEN, Hilden, Germany) or the FastDNA kit (Bio 101, Carlsbad, Calif.). DNA was amplified using Taq DNA polymerase (Gibco BRL, Life Technologies) as previously described (12) but using the broad-range 16S rRNA gene primers 536f (5′-CAG CAG CCG CGG TAA TAC-3′) and 1050r (5′-CAC GAG CTG ACG ACA-3′). The sequences obtained from amplified DNA were compared with those available in GenBank using the BLASTN version 2.0 program through the website of the National Center for Biotechnology Information (2). PCR was also conducted on negative valve controls obtained from patients without IE, usually one control for every five patient samples. Positive amplification of any negative control caused the experiment to be considered unreliable. Only DNA corresponding to the bacterium responsible for the episode of IE was considered.

Organisms identified as the recently reclassified Streptococcus equinus, Streptococcus gallolyticus, Streptococcus infantarius, Streptococcus lutetiensis, and Streptococcus alactolyticus (32, 41) were regarded as "Streptococcus bovis" in our study.

Histopathology. One histopathologist (H.L.) examined all hematoxylin-eosin-stained sections of the valves and classified lesions as (i) consistent with IE, (ii) having no signs of IE, or (iii) indeterminate according to criteria defined elsewhere (21). Specific stains, such as Gram and Whartin-Starry, were used when necessary (21).

Statistical analysis. Demographic and clinical data, etiological agents (blood and valve culture results and serology), and duration of antibiotic treatments were compared using the χ^2 or Mann-Whitney test according to PCR results. Univariate and multivariate logistic regressions were used to assess the situations more likely to benefit from systematic PCR amplification of the 16S rRNA gene and sequencing of the valves. The variables evaluated were age, sex, duration of antibiotic treatment, positive blood cultures, and histology. STATA software (version 7.0) was used for analysis.

RESULTS

A total of 147 patients who underwent 156 valve replacements were studied. Table 1 summarizes the demographic and clinical features of the patients at the time of valve replacement surgery. The patients were receiving antibiotic treatment for IE when 126 of the valve replacements were performed, and bacterial DNA was amplified more frequently in these patients (76 of 126; 60%) than in patients who had completed antibiotic treatment for IE at the time of valve replacement surgery (11 of 30; 37%; P = 0.02). The median ages of patients with positive and negative PCR results were 58 (interquartile range, 42 to 70) and 60 (interquartile range, 50 to 69) years, respectively. Seventy-one percent of patients with positive PCR results and 70% of patients with negative PCR results were males.

To determine the effect of current antibiotic treatment on positive PCR results, we analyzed the data on 126 patients with IE who were receiving antibiotic treatment at the time of
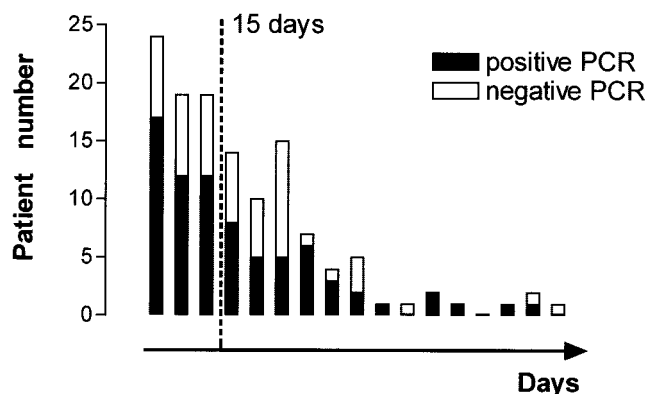
FIG. 1. Number of patients with a positive or negative PCR according to the delay between the beginning of antibiotic treatment and surgery (clusters of 5 days) among 126 episodes of valve replacement.

TABLE 3. Long-term persistence of bacterial DNA in patients who had completed antibiotic treatment for IE at the time of surgery[a]

Case/age/sex[b]	Bacterium	Involved valve	Delay between diagnosis and surgery
1/55/M	*Staphylococcus pneumoniae*	Aortic bioprosthesis	7 yr
2/69/F	*S. bovis*	Aortic, native	167 days
3/80/M	*S. bovis*	Mitral, native	730 days
4/39/M	*Enterococcus faecium*	Aortic bioprosthesis	850 days
5/36/M	*Streptococcus gordonii*	Aortic, native	45 days
6/33/M	*Bartonella quintana*	Aortic homograft	224 days
7/70/F	*Streptococcus sanguinis*	Mitral bioprosthesis	545 days

[a] All patients had no clinical or histological signs of IE, and valve cultures were sterile.
[b] M, male; F, female.

surgery. The median duration of treatment was 19.5 days (range, 1 to 150) for patients with negative PCR results and 14 days (range, 1 to 79) for those with positive PCR results ($P = 0.28$). The median duration of antibiotic treatment for all of the patients was 15 days. Consequently, we analyzed as a whole and separately patients with >15 and ≤15 days of antibiotic therapy. PCRs were positive for 66% of patients receiving antibiotics for ≤15 days and for 55% of patients receiving >15 days of antibiotic treatment ($P = 0.19$) (Fig. 1).

In this group of 126 patients with IE who were receiving antibiotic treatment at the time of surgery, *S. bovis*, oral viridans streptococci, and enterococci accounted for 21, 18, and 13% of cases of IE, respectively. *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) represented 17 and 10% of the cases of IE, respectively. *Bartonella* spp. accounted for 5% of the cases of IE. Table 2 shows the PCR results for each of the species causing IE in the study. Valves infected with *Streptococcus* spp. were more likely to be positive by PCR than those infected with other species ($P = 0.047$). We detected bacterial DNA in all the valves of patients with *Bartonella* sp. IE (6 of 6). Valves infected with CNS were more likely to be negative by PCR than those infected with other species ($P = 0.02$). This association was confirmed by multivariate analysis adjusted for age, sex, duration of antibiotic treatment, and positive blood cultures (odds ratio = 0.26; 95% confidence interval, 0.07 to 0.95). Valves infected with streptococci were more likely to be positive by PCR (38 of 54) than those infected with CNS (4 of 13; $P = 0.01$) or with staphylococci and enterococci (26 of 51; $P = 0.04$).

Thirty patients with a history of IE who had completed their antibiotic treatment had valve replacement surgery. The median time between surgery and antibiotic therapy was 1,210 days (range, 53 to 9,125) in patients with negative PCRs and 167 days (range, 45 to 2,920) in patients with positive PCRs ($P = 0.01$). In 11 patients in this group, bacterial DNA was found in valve tissue a long time after the end of antibiotic treatment. Seven patients did not have any histological signs of IE at the time of surgery (Table 3), while four had histological signs of IE. These four patients with histological signs of IE were likely to still have evolutive IE, since they all had biological markers of inflammation and two of them had persistent echographic signs of IE. Among the seven patients with no histological signs of IE, two patients (patients 1 and 3) did not have any clinical, biological, or echographic signs of active endocarditis. None of these patients developed signs of IE after valve replacement.

Bacterial DNA was amplified by PCR in 67% (64 of 95) of valves with histological lesions indicating IE and in 38% (21 of 55) of valves that had no histological evidence of IE ($P = 0.001$). This significant correlation was confirmed by multivariate analysis adjusted for age and sex (odds ratio = 3.3; 95% confidence interval, 1.61 to 6.80). PCR amplification was positive in 74% (43 of 58) of valves in which bacteria were seen with Gram staining and in 44% (37 of 84) of valves that had no evidence of bacteria with Gram staining ($P < 0.001$). This significant correlation was also confirmed by multivariate analysis adjusted for age and sex.

TABLE 2. Comparison of results of PCRs for one species versus all species performed on heart valves from 126 patients with IE with antibiotic treatment at the time of surgery

Species	Positive PCR for species/ total positive PCR (%)	Negative PCR for species/ total negative PCR (%)	*P* value
Streptococcus spp. and enterococci	47/76 (62)	24/50 (48)	0.13
Streptococcus spp.	38/76 (50)	15/50 (30)	0.047
S. bovis	18/76 (24)	8/50 (16)	0.30
Oral streptococci	16/76 (21)	7/50 (14)	0.32
Enterococci	9/76 (12)	8/50 (16)	0.50
Staphylococcus spp.	17/76 (22)	17/50 (34)	0.15
S. aureus	13/76 (17)	8/50 (16)	0.87
CNS	4/76 (5)	9/50 (18)	0.02
Bartonella spp.	6/76 (8)	0	0.04
Others[a]	10/76 (13)	10/50 (20)	0.30

[a] Others: *Actinobacillus actinomycetans* (two cases), *Corynebaterium jeikeium*, *Corynebacterium diphteriae*, *Proteus mirabilis*, *Haemophilus* spp. (two cases), *Streptococcus pneumoniae*, *Proprionobacterium acnes*, *Micrococcus* spp., *Enterobacter cloacae* (two cases), *E. coli* (two cases), *Streptococcus* β *haemolytic* (three cases), *Streptococcus agalactiae*, *Neisseria sicca*, *rastonia*.

DISCUSSION

In our study, we included only patients who had IE with known bacterial etiology and who underwent surgery while receiving antibiotic treatment or after successful antibiotic treatment of the condition.

We found no significant correlation between the duration of antibiotic treatment and PCR results in patients receiving antibiotics at the time of surgery. The use of PCR amplification to monitor treatment of infectious disease has become a subject of interest. In an experimental model of systemic candidiasis, PCR results correlated with the therapeutic efficacy of antifungal treatment (45). In a rabbit syphilis model, PCR amplification results suggested that in most instances the presence of *Treponema pallidum* DNA reflects living organisms (46). In infected mice, *Borrelia burgdoferi* DNA quickly disappeared from tissues that became culture negative after antibiotic treatment (24). These studies suggest that bacterial DNA is cleared from the tissue sites of animals after bacterial death but that the DNAs from different pathogens may have different kinetics at different sites. In human pathology, molecular methods based on DNA detection have been used to monitor the treatment of Whipple's disease (34), Lyme arthritis (33), and infections with *Helicobacter pylori* (42) and *Chlamydia trachomatis* (1). Our results show that even though bacterial DNA seems to be cleared over time in patients with IE, DNA detection should not be used for monitoring antibiotic efficacy in patients with ongoing IE.

When we considered bacterial species separately, all patients with endocarditis due to *Bartonella* spp. were positive by PCR. This may reflect greater amounts of DNA in infected valves or the fact that DNA of *Bartonella* spp. remains for longer periods in tissues than is the case with DNA of other bacteria. Patients with IE due to *Streptococcus* spp. were more likely to have a positive PCR. This shows that DNA of *Streptococcus* spp. persists longer than DNA of other species. On the other hand, patients infected with CNS were more likely to have a negative PCR, indicating that DNA of these species is less persistent than DNA of other species.

Our study showed that PCR was more likely to be positive in patients whose histology was indicative of IE and when bacteria were observed in histological preparations. These findings are in agreement with previous reports that molecular detection and sequencing were useful in cases of IE that were culture negative due to prior administration of antibiotics (4, 11, 18, 19, 28, 47), particularly when histopathology revealed the presence of bacteria (10, 47).

In previous studies, PCR was carried out only on valves from patients with acute IE and those with histological evidence of IE (4, 10). We were interested in patients under treatment for IE and those with a past history of IE who had completed their antibiotic treatment at the time of surgery. PCR amplification was positive despite the absence of histological signs of IE in 7 of 30 patients with a past history of IE. Two of these, one previously reported by Branger et al. (5) without clinical, laboratory, or echographic signs of active IE, had evidence of prolonged persistence of bacterial DNA in their heart valves. Both live and dead bacteria may result in positive PCR assays (17, 25). Despite negative bacterial cultures, the presence of bacterial DNA in clinical specimens has been shown for prolonged periods in septic arthritis (6, 44), pulmonary tuberculosis (14, 49), and leptospirosis (3, 27). The significance of the persistence of DNA for long periods after antibiotic treatment is uncertain (17, 25). Morris et al. found that in some patients with IE that had apparently been cured, valve cultures were sterile and had no histological signs of IE, although they were positive for organisms with Gram staining (29). Positive PCRs and Gram stains may represent results from previous episodes of IE that have resolved, and the results of both these tests must be interpreted with care. Gauduchon et al. reported a case of IE with blood cultures yielding *Escherichia coli* (9). Six months previously, however, the patient had been diagnosed with IE due to *Streptococcus mutans*. During this second episode of IE, the patient underwent valve replacement. Histology showed gram-positive cocci in the valve, and PCR amplification and sequencing detected *S. mutans* DNA. The authors considered the patient to have recurrent IE due to *S. mutans* rather than a new infection with *E. coli*. This interpretation is challenged by our finding that streptococcal DNA may persist long after IE is cured.

In conclusion, our results show that as the time between successful treatment of IE and valve surgery increases, the likelihood of bacterial DNA being demonstrated in heart valves decreases. This indicates that bacterial DNA is cleared over time but that this might be a very slow process. Since there is no correlation between the duration of antibiotic treatment and PCR results in patients still on antibiotic treatment at the time of surgery, DNA detection should not be used as a tool for monitoring treatment in IE. The persistence of DNA does not necessarily correspond to the persistence of viable bacteria and therefore to the successful treatment of the infection.

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