Bacteremia after Endodontic Procedures in Patients with Heart Disease: Culture and Molecular Analyses

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Abstract

Introduction: Infective endocarditis (IE) is still associated with high mortality, and antibiotic prophylaxis strategies are under intense debate. We evaluated the incidence of bacteremia after root canal preparation in teeth with necrotic pulps and apical periodontitis. Methods: Blood samples were taken before and 5 and 30 minutes after endodontic treatment in teeth with apical periodontitis from individuals at high (n = 21) or no risk (n = 11) for IE. The former received prophylactic antibiotic therapy. Bacteriologic samples were taken from root canals before chemomechanical preparation to confirm pulp infection. Samples were subjected to aerobic and anaerobic culture and quantitative real-time polymerase chain reaction (qPCR), the latter to determine the total bacterial and streptococcal levels. Results: Culture revealed no bacteremia in all individuals. Analysis by qPCR showed that bacterial DNA occurred in all root canal samples. qPCR showed a similar incidence of bacteremia between patients who received or did not receive prophylactic antibiotic therapy (P > .05). In blood samples taken 5 minutes after endodontic procedures, bacteria were detected in 2 of 11 (18%) individuals not taking antibiotics and in 4 of 21 (19%) patients under prophylaxis. After 30 minutes, the incidence of bacteremia decreased to 2 of 21 (10%) in patients taking antibiotics and was undetectable in patients at no risk of IE. The incidence of bacteremia by streptococci was identical as that for total bacteria. Conclusions: No detectable bacteremia was evident by culture after treatment of infected root canals. Molecular analysis revealed bacterial DNA and streptococci in blood from some patients without a significant difference between individuals receiving or not receiving antibiotic prophylaxis. (J Endod 2016;42:1181–1185)

Key Words

Bacteremia, endodontic treatment, infective endocarditis, prophylactic antibiotic therapy

Infective endocarditis (IE) is a disease characterized by microbial colonization of the endothelial surface of the heart, prosthesis, or implantable cardiac devices. Its characteristic lesion (vegetation) consists of an amorphous mass of platelets and fibrin, with colonies of microorganisms and inflammatory cells. Despite advances in diagnosis and treatment, IE is still responsible for noticeably high rates of morbidity and mortality, and predisposed patients should be evaluated for antibiotic prophylaxis whenever they undergo medical and dental procedures that may cause bacteremia.

There are not many studies evaluating the incidence of bacteremia after endodontic treatment procedures. Baumgartner et al (5, 6) reported that nonsurgical root canal treatment resulted in a lower incidence of bacteremia (3%, as a result of overinstrumentation) than surgical flap reflection (83%), periradicular surgery (33%), and tooth extraction (100%). The incidence of bacteremia has been shown to be greater when endodontic instruments are used beyond the apical foramen (7, 8). Debelian et al (7) observed the occurrence of bacteremia in 54% of the cases after instrumentation 1 mm beyond the apical foramen when compared with 31% when instrumentation was 1 mm short of the apical foramen. A similar incidence of bacteremia after instrumentation short of the apex was shown by Savarrio et al (9). By causing bacteremia, endodontic treatment might increase the risk for occurrence of IE in patients with predisposing valve conditions (10).

Although most previous studies used culture for bacterial detection and identification (6–8), the low sensitivity and inability of this method to detect the presence of difficult-to-grow or as-yet-uncultivated bacteria may lead to underestimation of the real incidence of bacteremia. Molecular methods have been shown to be more accurate and reveal a more diverse bacterial population during bacteremia than culture (11).
Furthermore, previous studies evaluated the incidence of bacteremia, but the bacterial load, another important factor that may determine the outcome of bacteremia, has not been assessed.

The study of bacteremia after dental procedures is crucial to the development of IE prophylaxis guidelines, but few studies investigated the occurrence of bacteremia after endodontic treatment procedures. In addition, no previous study has investigated the magnitude of bacteremia after endodontic intervention, which is information necessary to infer the possible systemic repercussion of bacteremia. The aim of the present study was to evaluate the incidence of bacteremia after endodontic intervention, which is information necessary to establish cardiac risk factors for IE (12, 13). Patients considered as having IE with mitral regurgitation, and bicuspid or degenerative aortic valve disease (12) are at higher risk for IE. In Brazil, rheumatic valve disease is among the most prevalent cardiac risk factors for IE (12, 13). Patients considered as having a high or insignificant risk for IE. Thirty-two samples were collected from 27 patients (16 men and 11 women) with a mean age of 52 years (ranging from 30–75 years). The patients were distributed as follows: 18 patients with valvular heart disease and high risk for IE requiring prophylactic antibiotic therapy and 9 patients with coronary artery disease, considered as having no significant risk for IE and not requiring prophylactic antibiotics. All patients were referred for endodontic treatment of teeth with necrotic pulps, which was confirmed by pulp sensibility tests and radiographic evidence of apical periodontitis. All teeth were asymptomatic. Multiradical teeth, teeth with vital pulps, teeth with apical periodontitis lesions, teeth previously subjected to endodontic treatment, and patients with periodontal pockets deeper than 4 mm were excluded from the study. Three patients contributed 2 teeth each, and 1 patient contributed 3 teeth. The time elapsed between each treatment in the same patient was at least longer than 5 months. In short, 32 teeth with necrotic pulps and apical periodontitis were included in the study; 21 from patients at risk for IE and 11 from patients considered to be at no risk for IE.

Prophylactic Antibiotic Therapy

Prophylactic antibiotic therapy consisted of 2 g amoxicillin 1 hour before the endodontic intervention as recommended by the Brazilian Cardiology Society guidelines for patients at risk for IE (12). These guidelines extend the indication of prophylactic antibiotics to patients presenting with rheumatic heart valve disease, mitral valve prolapse with mitral regurgitation, and bicuspid or degenerative aortic valve disease (12). In Brazil, rheumatic valve disease is among the most prevalent cardiac risk factors for IE (12, 13). Patients considered as having no significant risk for IE did not receive prophylactic antibiotics.

Endodontic Procedures and Sample Collection

Before endodontic treatment, the oral cavity was rinsed with a solution of 0.12% chlorhexidine digluconate for 1 minute. Each tooth was isolated with a rubber dam, and the operative field was cleaned with 3% hydrogen peroxide and disinfected with 2.5% sodium hypochlorite (NaOCl). The access cavity was prepared with sterile burs, and the tooth, clamp, and rubber dam were once again disinfected with 2.5% NaOCl. Negative control samples were taken from the disinfected tooth crown using sterile paper points and evaluated by qPCR using universal 16S ribosomal RNA gene-based primers (see later). For inclusion in the study, these cases had to show negative results for bacteria. Next, samples were taken from the root canal using sequentially 3 sterile paper points placed up to 1 mm short of the apex as determined by radiographs. Each paper point remained in the canal for 1 minute and was then transferred to cryotubes containing Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and immediately stored at −20°C until qPCR analysis was performed.

Cleaning and shaping of the root canal were performed with the ProTaper Universal System (Dentsply Maillefer, Ballaigues, Switzerland) up to the F3 instrument under 5.25% NaOCl irrigation. The working length was established 1 mm short of the radiographic apex. In sequence, an intracanal dressing with calcium hydroxide was placed, and the tooth was temporized. The root canal was obturated at a subsequent appointment.

Before blood sample collection, the skin on the site of the median cubital vein was disinfected with 2% chlorhexidine gluconate, and 20 mL blood was collected immediately before and 5 and 30 minutes after the endodontic intervention. The samples obtained were immediately processed for aerobic (9 mL) and anaerobic culture (9 mL), and 2 mL blood was stored at −80°C for further molecular microbiology analysis.

Blood Culture Analysis

Peripheral blood samples were cultured using the Bact/ALERT 3D system (BioMérieux Inc, Durham, NC). Blood was collected in bottles containing aerobic and anaerobic media and was incubated for 5 and 15 days, respectively. Bottles were monitored, and if bacterial growth was evident, they were subcultured onto 5% sheep blood agar plates supplemented with 5 µg/mL hemin (Sigma-Aldrich, St Louis, MO) and 1 mg/mL menadione (Sigma-Aldrich). Plates were incubated in aerobic, capnophilic (5% CO2) and anaerobic (85% N2, 5% H2, and 5% CO2) atmospheres for 48 hours at 37°C. For anaerobiosis, samples were handled and incubated inside an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI).

qPCR Analysis

Real-time qPCR using primers to the 16S ribosomal RNA gene was used to quantify the total bacterial load and the prevalence and levels of Streptococcus species in the root canal and blood samples. Analysis was performed in a total volume of 20 µL containing 2 µL DNA extracted from each clinical sample, primers (0.5 mmol/L each), and PowerSYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an ABI 7500 thermocycler (Applied Biosystems). The primers were as described previously (14, 15), and their sequences and annealing temperatures are listed in Table 1. Samples were dispensed into 96-well plates (MicroAmp Optical, Applied Biosystems), sealed, and centrifuged. qPCR was run according to the following settings: 95°C/10 minutes followed by 40 amplification cycles at 95°C/1 minute, annealing temperature (Table 1)/1 minute, and 72°C/1 minute. After each cycle, polymerase chain reaction (PCR) products were monitored for the increase in fluorescence of SYBR Green. All measurements were performed in triplicate for both samples and controls. For the negative control, ultrapure water was used replacing the clinical sample. To determine the specificity of the amplified products, a melting curve was obtained from 60°C to 95°C, with continuous fluorescence measurements for each 1% increase in temperature. Data acquisition and analyses were performed using the ABI 7500 software, version 2.0.6 (Applied Biosystems).

Bacterial counts were determined for each sample based on standard curves. The standard curve for both universal and Streptococcus
primes was generated using DNA extracted from known concentrations of Streptococcus mutans ATCC 25175 grown in pure culture. Knowing the size of the S. mutans genome (2 Mb) and the average molecular weight of 1 base pair (bp) (660 Da), the measured DNA value could then be converted into target genomic copy levels per microliter using the formula \( m = \frac{n(1 \text{ mole} / 6 \times 10^{25} \text{ [bp]})}{(660 \text{ [g/mole]})} = \frac{n(1.096 \times 10^{-21} \text{ [g/bp]})}{m} \), where \( m \) is the genomic mass of a single cell and \( n \) the genome size. The numbers of genomic copies were considered numerically equivalent to the levels of bacterial cells. The DNA standards were then 10-fold diluted from \( 10^2 \) to \( 10^5 \) cells in Tris-EDTA buffer and used to generate the standard curve.

**Statistical Analysis**

Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation or median and interquartile range as appropriate. Discrete variables were presented as percentages. Comparisons between groups were performed by unpaired Student t test, Mann-Whitney, or Fisher exact tests as appropriate. The level of significance was set at \( P < .05 \). Calculations were performed using the commercially available statistical software GraphPad Prism 3.02 (GraphPad Software Inc, San Diego, CA) and MedCalc 9.2.0.2 (MedCalc Software, Mariakerke, Belgium).

**Results**

**Blood Culture**

One blood sample taken before endodontic intervention showed aerobic growth of a Micrococcus species, which was considered a contaminant. All remaining blood samples collected before and after root canal procedures were negative for bacterial growth.

**qPCR Analysis**

Analysis by qPCR showed that bacterial DNA occurred in all root canal samples. Streptococcus species were detected in 6 of 34 (18%) canal samples. No significant differences were observed in the total bacterial and streptococcal counts in root canal samples between the 2 groups of patients (\( P > .05 \), Table 2).

Regarding the blood samples collected 5 minutes after endodontic procedures, qPCR with universal bacterial primers revealed an incidence of bacteremia of 19% (4/21) in patients at risk for IE and 18% (2/11) in patients with no risk of IE. After 30 minutes, the incidence of bacteremia decreased to 10% (2/21) in the patients at risk for IE and was not detected in the patients with no risk. The incidence of bacteremia by streptococci was identical to that detected by qPCR for total bacteria counts. No significant difference was observed in the incidence of bacteremia between patients who received prophylactic antibiotic therapy and those not treated with antibiotics (\( P > .05 \)). Counts of total bacteria and streptococci in samples with bacteremia are shown in Table 2. No significant difference was observed in the bacterial counts between the 2 groups studied (Table 2).

Discussion

Several authors have evaluated the incidence of bacteremia after different dental procedures, most of them using conventional culture methods (6–8, 16). There has been a wide range of reported bacteremia depending on the dental procedure and the studied population. The frequency of positive cultures ranged from 58%–100% after dental extractions, from 8%–79% after periodontal surgery, from 10%–70% after tooth brushing, and from 3%–40% after tooth brushing (4, 16–22). On the other hand, the reported incidence of IE after dental procedures is low or difficult to estimate, and the risk of antibiotic overuse may overcome the benefit of antibiotic prophylaxis. Therefore, the American Heart Association published international guidelines with strict indications for prophylactic antibiotic therapy for patients undergoing medical and dental procedures (3). The Brazilian Cardiology Society follows the same guidelines but also recommends prophylactic antibiotics for patients with rheumatic valve diseases, mitral valve prolapse with mitral regurgitation, and degenerative or bicuspid aortic valve disease (12). These recommendations were followed in the present study.

In this study, bacteremia was not detected by culture 5 and 30 minutes after endodontic treatment in both groups (receiving or not systemic antibiotic prophylaxis). This finding is in agreement with Bender et al (8), who found no bacteremia after instrumentation short of the foramen, but diverges from other culture studies that reported frequencies of bacteremia ranging from 3.3%–31% (6–7, 9). However, data from qPCR analysis revealed an incidence of bacteremia within the range of the latter studies when evaluated 5 minutes after endodontic treatment. After 30 minutes, the incidence of bacteremia decreased in both groups. The bacterial load and number of streptococci in the root canal and the incidence of bacteremia by streptococci detected by qPCR after endodontic treatment were similar between patients from both groups. Because bacteremia was not detected in the 2 groups evaluated by culture and antibiotic prophylaxis did not change the frequency of bacteremia as determined by qPCR, it is reasonable to put into question the need of antibiotic prophylaxis before endodontic intervention, especially from a risk/benefit standpoint. On the other hand, a previous study reported that antibiotic prophylaxis reduced the incidence of bacteremia and the number of species present in the blood stream after dental extraction (23). However, the same study showed that some species of proteobacteria and Prevotella were not significantly affected (23). Because complications from both IE and antibiotic overuse can be serious and life-threatening, further studies on the subject are required to form a solid body of evidence for decision making about antibiotic prophylaxis for patients at risk of IE.

The present study showed bacteremia only when using the molecular approach, and the greater incidence may be related to the lower detection limits (higher sensitivity) of the qPCR approach and its ability to detect difficult-to-grow and uncultivable bacteria that may pass unnoticed by culture (24). Moreover, molecular microbiology detection methods are not expected to be significantly affected by concomitant antibiotic therapy (25). Our findings are in agreement with a previous

**TABLE 1. Primers Used for Bacterial Detection and Quantification**

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer sequences</th>
<th>Annealing temperature (°C)</th>
<th>Fragment length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal 16S ribosomal RNA gene</td>
<td>5′-GAT TAG ATA CCC TGG TAG TCC AC-3′*</td>
<td>52</td>
<td>733</td>
<td>14</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>5′-TAC CTT GTT ACG ACT T-3′*</td>
<td>60</td>
<td>~120–130</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>5′-AGA TGG ACC TGC GTT GT-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5′-GCT GCC TCC CGT AGT CT-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Universal primer.
Clinical Research

TABLE 2. Levels of Total Bacteria and Streptococci in the Peripheral Blood and Root Canal from Patients at Risk or with No Risk for Infective Endocarditis (IE)

<table>
<thead>
<tr>
<th>Time</th>
<th>At risk for IE (n = 21)</th>
<th>No risk for IE (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood: total bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>$0 \times 10^2$</td>
<td>$1.42 \times 10^2$</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>$0-4.02 \times 10^2$</td>
<td>$0-1.32 \times 10^2$</td>
</tr>
<tr>
<td>Range</td>
<td>$0-1.6 \times 10^2$</td>
<td>$0-0$</td>
</tr>
<tr>
<td>Blood: streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>$0$</td>
<td>$0$</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>$0$</td>
<td>$0$</td>
</tr>
<tr>
<td>Range</td>
<td>$0-4.35 \times 10^2$</td>
<td>$0-1.0 \times 10^2$</td>
</tr>
<tr>
<td>Root canal: total bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>$8.01 \times 10^4$</td>
<td>$6.74 \times 10^4$</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>$9.15 \times 10^4$</td>
<td>$8.26 \times 10^4$</td>
</tr>
<tr>
<td>Range</td>
<td>$1.72 \times 10^5$–$3.16 \times 10^5$</td>
<td>$1.07 \times 10^5$–$2.43 \times 10^5$</td>
</tr>
<tr>
<td>Root canal: streptococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>$1.34 \times 10^4$</td>
<td>$8.91 \times 10^3$</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>$1.43 \times 10^4$</td>
<td>$1.00 \times 10^5$</td>
</tr>
<tr>
<td>Range</td>
<td>$0-2.52 \times 10^5$</td>
<td>$2.33 \times 10^5$–$1.89 \times 10^5$</td>
</tr>
</tbody>
</table>

Data from quantitative real-time polymerase chain reaction.

study that observed that molecular methods provide more accurate results in terms of bacterial detection in bacteremia after dental extractions when compared with culture (11). However, another study involving culture and molecular methodology (end-point PCR) for the detection of bacteremia after endodontic procedures reported 30% incidence by culture and a lower incidence by PCR (9). The reason for the difference when compared with the present study may be related to methodological issues, including the primers used, the method for DNA extraction from blood, and the higher sensitivity of the real-time qPCR approach in comparison with the end-point PCR method. The discrepancies between culture and molecular methods may put into question findings from previous studies about bacteremia after dental procedures and point to a need to combine and improve methods for more accurate results.

Another great advantage of the qPCR approach was to determine the magnitude of bacteremia, which has not been shown in previous studies related to endodontic procedures. For the bacteremia to cause systemic effects, factors other than the mere presence of bacterial cells in the bloodstream are of utmost importance. They include the virulence ability and the bacterial counts (load). In the present study, the mean level of total bacteria and streptococci in the bacteremic event (5 minutes) was in the order of $10^4$ to $10^5$/mL blood in both groups. These numbers are below the range of the inoculum of $10^4$ to $10^5$ bacterial cells/mL blood necessary to cause experimental IE in the animal model (26). The systemic effects of these levels of bacteria are unknown, but they will conceivably depend on the types of bacteria present and their virulence.

The duration of bacteremia can also influence its systemic effects. Bender et al (8) reported that bacteremia after endodontic procedures lasted no longer than 10 minutes as a result of competent clearance of bacteria from within the circulation. In this study, the incidence of bacteremia as detected by qPCR reduced with time, but even in the last examination period (30 minutes), 2 patients still exhibited qPCR-positive blood samples. These findings are in agreement with other reports of positive blood cultures detected for as long as 30 minutes after a dental procedure (17, 27–30). Actually, bacteremia has been observed to persist for at least 60 minutes after brushing and extraction in patients not receiving antibiotic prophylaxis (4). How much longer than 30 minutes bacteremia can persist after endodontic procedures was not possible to determine in the present study and should be evaluated further.

Advantages of the qPCR approach are recognized, but this approach also has limitations. DNA-based molecular methods can detect bacteria that are no longer viable (31). Therefore, one cannot discard the possibility that qPCR may have detected DNA from bacteria in the bloodstream that died shortly before analysis as a result of the antibiotic therapy or immune system response. These bacteria may have participated in the infectious process, but their detection is not relevant from a clinical perspective because they do not need to be targeted by antibiotics. Further investigations using molecular microbiology methods that circumvent this limitation of DNA-based approaches are necessary to determine whether the detection of bacteremia by the molecular method was because of the greater sensitivity of the technique, the ability to detect uncultivable bacteria, or the detection of DNA from dead cells.

In conclusion, this study showed that the incidence of bacteremia after endodontic treatment procedures varied from none (culture) to low occurrence (qPCR). In the positive cases, qPCR revealed low bacterial counts per milliliter of blood. These findings suggest that, for infected teeth from patients with a history of cardiac valve disease, endodontic therapy should be the treatment of choice because the alternative, extraction, has been associated with a greater incidence of bacteremia (4, 17). Antibiotic prophylaxis showed no apparent influence on the incidence and levels of bacteremia as determined by qPCR, and further studies are required to determine whether or not antibiotic prophylaxis is really necessary before endodontic intervention in patients at risk of IE.
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