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Periodontopathic microorganisms in peripheric blood after scaling and root planing

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Abstract

Aim: The objective of this study was to evaluate the frequency of periodontopathic and other subgingival anaerobic and facultative bacteria in the bloodstream following scaling and root planing (SRP).

Material and Methods: Forty-two patients with severe generalized chronic periodontitis (GChP) and generalized aggressive periodontitis (GAgP) were included in the study. Four samples of peripheric blood were drawn from the cubital vein at different times: Pre-treatment: immediately before the SRP procedure (T1), immediately after treatment (T2), 15 min. post-treatment (T3) and 30 min. post-treatment (T4). In order to identify the presence of microorganisms in blood, subcultures were conducted under anaerobic conditions.

Results: 80.9% of the patients presented positive cultures after SRP and it occurred more frequently immediately after treatment; however, 19% of the patients still had microorganisms in the bloodstream 30 min. after the procedure. The periodontopathic microorganisms more frequently identified were *Porphyromonas gingivalis* and *Micromonas micros. Campylobacter* spp., *Eikenella corrodens*, *Tannerella forsythensis*, *Fusobacterium* spp. and *Prevotella intermedia* were isolated less often. *Actinomyces* spp. were also found frequently during bacteraemia after SRP.

Conclusions: SRP induced bacteraemia associated with anaerobic bacteria, especially in patients with periodontal disease.

Key words: *Actinomyces* spp.; bacteraemia; cardiovascular disease; periodontal disease; *Porphyromonas gingivalis*; scaling and root planing

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Different epidemiologic studies have reported a risk association between periodontal disease and acute myocardial infarction and stroke (Mattila et al. 1989, 1993, 1995, De Stefano et al. 1993, Beck et al. 1996, Grau et al. 1997, Wu et al. 2001, Janket et al. 2003). Local infection and transient bacteraemia due to periodontopathic bacteria can be related to the pathogenesis of the association between periodontal disease and cardiovascular disease by different mechanisms (Beck et al. 1996, Offenbacher et al. 1999, Ebersole et al. 2002, Genco et al. 2002). The microorganisms more frequently associated with periodontitis are: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythensis*, *Eikenella corrodens*, *Campylobacter rectus*, *Micromonas micros*, *Treponema denticola, Fusobacterium nucleatum* and *Prevotella intermedia* (Newman & Socransky 1977, Loesche et al. 1985, Dzink et al. 1988, Socransky et al. 1998). Most of these microorganisms have been identified by molecular techniques in atheromas and may be associated with transient bacteraemia (Haraszthy et al. 2000, Ishihara et al. 2004, Cavrini et al. 2005, Fiehn et al. 2005, Ford et al. 2006, Kozarov et al. 2006, Padilla et al. 2006).

Recently, prospective studies have also provided serological evidence that infections caused by major periodontal

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pathogens like *A. actinomycetemcomitans* and *P. gingivalis* are associated with future stroke (Pussinen et al. 2004a) and that *P. gingivalis* also increases the risk of myocardial infarction (Pussinen et al. 2004b). Moreover, *P. gingivalis* may be a concomitant risk factor in the development of Acute Coronary Syndrome (ACS) (Renvert et al. 2006).

Transient bacteraemia has been reported in clinical trials after different preventive dental procedures and periodontal therapy. Bacteraemia has been reported after tooth brushing (Sconyer et al. 1973, Caroll & Sebor 1980, Schlein et al. 1991, Kinane et al. 2005, Forner et al. 2006a), chewing (Everett & Hirschman 1977, Forner et al. 2006a, b), periodontal probing (Daly et al. 2001, Kinane et al. 2005), subgingival irrigation (Waki et al. 1990), scaling (Heimdahl et al. 1990, Kinane et al. 2005, Forner et al. 2006a), scaling and root planing (SRP) (Lazansky et al. 1949, Conner et al. 1967, Messini et al. 1999), periodontal surgery (Lockhart 1996) and dental extractions (Everett & Hirschman 1977, Head et al. 1984, Heimdahl et al. 1990, Vergis et al. 2001). However, the results showed considerable variability due to the techniques used, timing of blood sample collection, periodontal status and identification methods for the isolation of microorganisms.

There are few well-conducted studies that have determined the frequency of passage of periodontal microorganisms to the bloodstream after periodontal treatment. They have evaluated this process only after scaling (Heimdahl et al. 1990, Kinane et al. 2005, Forner et al. 2006a). Root planing in addition to scaling is the procedure most used in periodontal therapy and maintenance in patients with periodontitis and may be a risk factor for bacteraemia. However, there is scarce information related to the incidence of periodontopathic microorganisms during bacteraemia induced by this procedure. The aim of this study was to establish the frequency of passage of periodontopathic microorganisms in peripheric blood after SRP in patients with periodontitis.

Materials and Methods

Study population

Forty-two patients, 27 with a diagnosis of generalized severe chronic periodontitis (GChP) and 15 with a diagnosis of generalized aggressive periodontitis (GAgP) who attended the Graduate Clinic of Periodontics and Oral Medicine at the Universidad El Bosque in Bogotá, Colombia, participated in the study. The study was conducted after approval by the Institutional Review Board of the Universidad El Bosque, and all patients agreed to participate on a voluntary basis and signed a consent form.

Inclusion and exclusion criteria

Patients were diagnosed following the criteria established by the American Academy of Periodontology (AAP) in 1999 (Armitage 1999). Clinical history and radiographic examination were conducted for each patient and laboratory exams such as a glucose test and a haemogram were requested in order to exclude medically compromised patients. Subjects with the following conditions were excluded: congenital valve defects or any other risk situation for infectious endocarditis, low levels of haematocrit and/or haemoglobin, high risk of cardiovascular disease, diabetes and patients who had taken systemic antibiotics 1 month before the procedure. The periodontal examination included the assessment of pocket depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP) of all the sites clinically examined. Gingival index (GI) (Löe & Silness 1963) and PD of the treated sites with SRP were also determined. Patients must have had at least 10 pockets with probing depth $\geqslant$7 mm requiring periodontal surgery after SRP.

Clinical protocol

Four blood samples were drawn from each patient at the following times: Pre-treatment: immediately before the SRP procedure (T1), immediately after treatment (T2), 15 min. after treatment (T3) and 30 min. after treatment (T4).

On the day of the blood sample collection, patients were requested not to brush their teeth before the appointment and to consume only liquids during breakfast. In order to standardize the time of each one of the procedures in all the patients, SRP was conducted for 1 min. for each site in each of the 10 selected sites for a total of 10 min./patient. All the mechanical procedures were performed by the same operator (G. L.).

Blood sampling procedures

Procedures were conducted in a surgery room in order to provide a sterilized environment and to avoid any kind of contamination. Before initializing the procedure, cannulation of the vein was performed in the following manner: after skin preparation with 1% Povidone-Iodine solution, cannulation of the cubital vein was performed by an expert nurse using a 18GA IV catheter (BD Insyte, Becton Dickinson, NJ, USA). An Injection Site Adapter (Becton Dickinson) was then positioned and attached to a Sterile Multiple Sample Needle (Vacutainer, Becton Dickinson). Five millilitres of blood was then drawn in order to obtain the pre-treatment blood sample. Immediately after the pre-treatment sample was collected and the SRP procedure was finished, post-treatment blood samples (5.0 ml each) were taken (immediately, 15 and 30 min. later). Blood samples were drawn through the needle into a bottle containing 50 ml of Ruiz-Castañeda biphasic medium (Bio-Bacter, Bogotá, Colombia) consisting of one solid phase (trypticase soy agar and agar-agar) and one liquid phase (trypticase soy broth, SPS and sucrose) enriched with haemin (0.0005%) and menadione (0.00005%).

Blood culture bottles were incubated for 15 days at 36°C and monitored every day for the presence of bacteria. Subcultures were conducted as soon as growth was observed. Negative cultures were processed in the same manner as the positive cultures after 7 and 15 days of incubation before discarding the sample.

For the identification of *P. gingivalis*, *T. forsythensis*, *Campylobacter* spp., *E. corrodens* and other anaerobic and facultative microorganisms, 100 μl of the blood culture bottle were inoculated in Brucella agar (BBL Microbiology Systems, Cockeysville, MD, USA) enriched with 0.3% Bacto Agar, 0.2% yeast extract, 5% defibrinated sheep blood, 0.2% haemolysed sheep red blood cells, 0.0005% haemin and 0.00005% menadione, and incubated in anaerobic atmosphere with 9–13% CO_2 and an oxygen concentration below 1% (Anaerogen, Oxoid, Hampshire, UK) at 36°C for 7 days. For identification of *A. actinomycetemcomitans*, samples were plated onto a TSBV (Slots 1982) selective medium (4% Tryptone Soy Agar, 0.1% yeast extract, 10% horse serum, 75 μg/ml bacitracin and 5 μg/ml vancomycin) and incubated at 36°C for

3–5 days in 10% CO_2 atmosphere (Campygen, Oxoid). Bacteria were identified based on the colonies morphology, Gram stain and the following specific biochemical tests: ultraviolet (UV) light test (Slots & Reynolds 1982) and CAAM test (Slots 1987) for *P. gingivalis*, CAAM test for *T. forsythensis* and nitrite and oxidase tests for *E. corrodens*. *A. actinomycetemcomitans* was recognized by the presence of a star-like structure within the colonies in TSBV agar and by the MUG (Alcoforado et al. 1987) and catalase tests. API-ZYM and RAPID ID 32A systems (Biomerieux, Lyon, France) were also used for confirming the species of periodontopathic and other microorganisms growing in an anaerobic atmosphere.

Statistical analysis

Data were entered in a Microsoft Excel spreadsheet and then processed in MINITAB 14.0. (Minitab Inc., State College, PA, USA). Descriptive analyses were conducted to assess the distribution of the different clinic's variables in the groups studied (frequency distribution for categorical variables and mean and standard deviation for continuous variables). Descriptive analyses also involved the frequency distribution of microorganisms observed in blood at different times. χ^2/Fishers exact tests were used to compare the incidence of bacteraemia between groups (GChP or GAgP) and the microorganisms isolated in both groups. Statistical significance was set at a p-value < 0.05.

Results

Sample characteristics

Table 1 depicts the sociodemographic aspects of the studied sample. There were significant differences between the groups ($p < 0.05$). Patients with GChP were older than patients with GAgP. Analyses of clinical indicators were person-based expressed as the average of all the sites clinically examined. There were significant differences in all the clinical parameters between patients with GChP and GAgP ($p < 0.05$) but not for bleeding on probing. GAgP patients showed a higher probing depth (of sites selected for SRP): $p < 0.05$, but the GI was more severe in GChP ($p < 0.05$).

Frequency of microorganisms in blood cultures after SRP

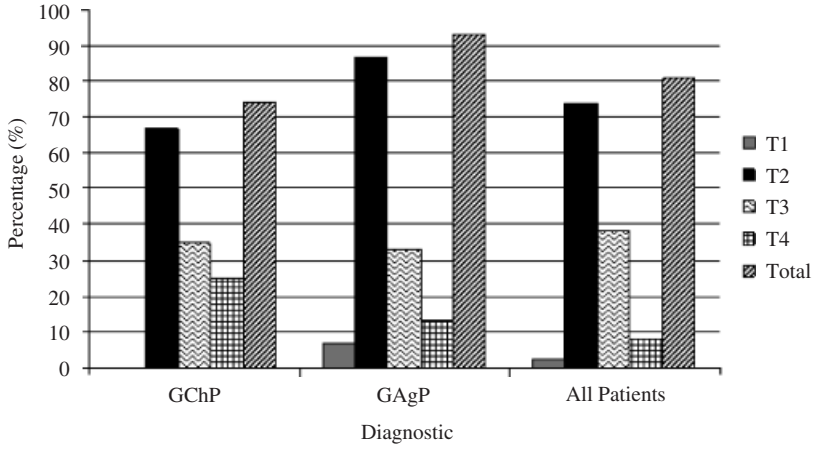
Microorganisms growing under anaerobic conditions were identified in the peripheric blood of 80.9% (34/42) of the patients after SRP. Bacteraemia was found in 93.7% (14/15) of the GAgP patients and 74.1% (20/27) of the GChP patients without significant differences between the two groups of periodontitis ($p > 0.05$). In 73.8% (31/42) of the patients, bacteria were isolated in blood immediately after treatment (T2). In 38% (16/42) of the patients, bacteraemia was evident after 15 min. (T3), and 19% (8/42) still had positive cultures after 30 min. (T4). One patient (2.4%) presented a positive culture before SRP (T1) (Fig. 1).

The periodontopathic microorganisms more frequently found in peripheric blood were *P. gingivalis*, and *M. micros*. *Campylobacter* spp., *E. corrodens*, *T. forsythensis*, *Fusobacterium* spp. and *P. intermedia/nigrescens* were isolated less frequently. *Actinomyces* spp. was isolated frequently and *Capnocytophaga* spp., *Prevotella melaninogenica*, *Propionibacterium acnes, Bifidobacterium* spp., *Eubacterium aerofaciens* and *Gemella*

Table 1. Age, gender and clinical parameters in patients treated with scaling and root planing

Parameter	Generalized chronic periodontitis (GChP)		Generalized aggressive periodontitis (GAgP)	
	total	%	total	%
Subjects	27/42	64.28	15/42	35.72
Gender (%)				
F	15	55.5	10	66.7
M	12	44.5	5	33.3
Age (mean ± SD)	42.9 ± 8.3*		33.4 ± 4.9	
PD (mean ± SD)	3.7 ± 0.9		4.4 ± 0.8*	
CAL (mean ± SD)	3.7 ± 1.4		5.0 ± 1.1*	
Bleeding on probing (% positive)	62.8 ± 26.8		73.9 ± 22.9	
PD-treated sites with SRP mm (mean ± SD)	7.4 ± 2.0		8.3 ± 1.8*	
GI-treated sites with SRP (% positive sites for each degree)	Grade 1 = 30.5% Grade 2 = 44.0% Grade 3 = 25.5%*		Grade 1 = 31.0% Grade 2 = 57.0% Grade 3 = 12.0%	

PD, probing depth; CAL, clinical attachment level; GI, gingival index (Löe & Silness 1963).
*$p < 0.05$ by t-test or χ^2/Fishers exact test.



T1: Pre-treatment
T2: Immediately after treatment
T3: 15 minutes after treatment
T4: 30 minutes after treatment
Total: Positive at any time after scaling and root planning

Fig. 1. Incidence of microorganisms in patients with a positive blood culture after scaling and root planing at different times of evaluation.

morbillorum were also isolated but in lower proportion. *P. gingivalis*, *Actinomyces* ssp., *T. forsythensis* and *P. intermedia/nigrescens* were isolated more frequently from the blood of GAgP patients than GChP patients but the differences were significant only for *T. forsythensis* ($p<0.05$) (Table 2). Table 3 shows the patients who showed positive blood cultures at the different evaluation times (T1, T2, T3 and T4) and the periodontopathic microorganisms isolated in each case.

Table 2. Frequency of microorganisms in blood cultures after scaling and root planing

Microorganism	GChP [$n=27$ F (%)]	GAgP [$n=15$ F (%)]	Total [$n=42$ F (%)]
Porphyromonas gingivalis	6 (22.2)	6 (40)	12 (28.6)
Actinomyces spp.	5 (18.5)	7 (46.6)	12 (28.6)
Micromonas micros	4 (14.8)	3 (20)	7 (16.7)
Campylobacter spp.	3 (11.1)	2 (13.3)	5 (11.9)
Capnocytophaga spp.	4 (14.8)	1 (6.6)	5 (11.9)
Fusobacterium spp.	4 (14.8)	1 (6.6)	5 (11.9)
Prevotella melaninogenica	4 (14.8)	1 (6.6)	5 (11.9)
Propionibacterium acnes	4 (14.8)	1 (6.6)	5 (11.9)
Eikenella. corrodens	4 (14.8)	0 (0)	4 (9.5)
Tannerella forsythensis	1 (3.7)	2 (13.3)	3 (7.1)
Prevotella intermedia/nigrescens	0 (0)	3 (20)	3 (7.1)
Bifidobacterium spp.	2 (7.4)	0 (0)	2 (4.8)
Eubacterium aerofaciens	1 (3.7)	0 (0)	1 (2.4)
Gemella morbillorum	1 (3.7)	0 (0)	1 (2.4)

GChP, generalized chronic periodontitis; GAgP, generalized aggressive periodontitis.

Discussion

The evaluation of bacteraemia after SRP has been analysed by Lazansky et al. in 1949 and Conner et al. in 1967 who isolated only aerobic Gram-positive bacteria. Previous studies did not report the presence of anaerobic and facultative bacteria, perhaps due to the lack of accurate procedures for isolating and identifying this kind of microorganisms. The objective of this study was to evaluate the passage of periodontal bacteria to the bloodstream after SRP in order to know the incidence of bacteraemia induced for these microorganisms in periodontitis patients. Aerobic bacteria were not considered in this study.

Messini et al. (1999) conducted a study investigating the initial time and duration of bacteraemia in disabled patients after dental treatment under general anaesthesia including SRP. They found 83% aerobic and anaerobic bacteria. Recently, Kinane et al. (2005) on analysing patients with periodontitis, reported a frequency of 23% by polymerase chain reaction (PCR) (using universal bacterial primers that target the 16S ribosomal RNA gene) and 13% by culture of aerobic and anaerobic bacteria after full-mouth ultrasonic scaling. The incidence of bacteria was lower

Table 3. Periodontopathic microorganisms isolated from GChP and GAgP patients in blood cultures at different times

Patient	Diagnostic	T1	T2	T3	T4
1	GAgP		*Porphyromonas gingivalis*		
2	GChP		*Eikenella corrodens*	*E. corrodens*	*E corrodens*
8	GAgP				*P. gingivalis*
9	GChP		*P. gingivalis* *Campylobacter* spp.	*P. gingivalis*	
12	GAgP			*P. gingivalis*	
13	GChP		*P. gingivalis*		
15	GAgP		*Micromonas micros*	*M. micros*	*M. micros*
18	GChP			*M micros*	
19	GChP		*M. micros*		
20	GChP		*P. gingivalis*	*P. gingivalis*	
27	GChP			*P. gingivalis* *Tannerella forsythensis*	*P. gingivalis*
28	GAgP		*P. gingivalis*	*P. gingivalis* *M. micros*	
30	GChP		*E. corrodens*	*P. gingivalis*	
31	GAgP	*Campylobacter* spp. *Prevotella intermedia/nigrescens*			
32	GAgP		*T. forsythensis* *P. intermedia/nigrescens*		
33	GAgP		*P. gingivalis*		
34	GAgP			*T. forsythensis*	
36	GAgP		*P. gingivalis* *P. intermedia/nigrescens*		
37	GChP		*Campylobacter* spp. *E. corrodens*		
38	GAgP		*Campylobacter* spp.		*M. micros*
40	GChP		*M. micros* *Campylobacter* spp.	*P. gingivalis* *E. corrodens*	

T1, Pre-treatment; T2, Immediately after treatment (1 min.); T3, 15 min. after treatment; T4, 30 min. after treatment; GChP, generalized chronic periodontitis; GAgP, generalized aggressive periodontitis.

than that observed in the current study (80.9%). Forner et al. (2006a, b) found 75% aerobic and anaerobic bacteria in periodontitis patients after scaling. Their results are lower than the percentage observed in this study, considering that we only evaluated anaerobic and facultative microorganisms. These results suggest that SRP could represent a higher risk of bacteraemia associated with periodontopathic microorganisms in periodontitis patients.

In the present study, the frequency of bacteraemia was slightly higher in GAgP patients than in GChP patients. A positive but weak correlation between the level of gingival inflammation and the presence of microorganisms in blood after scaling was reported by Forner et al. (2006b). In this study, a higher frequency of bacteraemia was observed in the GAgP group, which also showed lower levels of gingival inflammation than GChP patients. *P. gingivalis*, *Actinomyces* ssp., *T. forsythensis* and *P. intermdia* were higher in GAgP than GChP. This observation may suggest the existence of other mechanisms involved in the presence of bacteraemia that should be studied further.

In the current study, other microorganisms different from those reported by Kinane were isolated. *P. gingivalis, Actinomyces* spp. and. *M. micros* were the microorganisms more frequently isolated in peripheric blood, followed by *Campylobacter* spp., *Fusobacterium* spp., *Capnocytophaga* spp., *E. corrodens*, *T. forsythensis* and *P. intermedia*. Kinane et al. (2005) reported the presence of bacteria after scaling but very few genera were associated with periodontal disease. Although Forner et al. (2006a) recovered *P. gingivalis*, *Fusobacterium* spp. and *P. intermedia* in blood. The frequency of *P. gingivalis* was lower than our findings perhaps because we induced bacteraemia after SRP in patients with severe periodontitis.

P. gingivalis showed the highest frequency of isolation in peripheric blood after SRP. This microorganism has also been isolated frequently from atheromas which may represent an additional risk for the development of vascular lesions (Haraszthy et al. 2000, Stelzel et al. 2002, Ishihara et al. 2004, Cavrini et al. 2005, Ford et al. 2006). A study conducted in animals showed that recurrent *P. gingivalis* bacteraemia induces aortic and coronary lesions consistent with atherosclerosis in normocholesterolaemic pigs and increases aortic and coronary atherosclerosis in hypercholesterolemic pigs (Brodala et al. 2005). *P. gingivalis* has been evaluated also by its capability to induce vascular changes by different mechanisms: invasion of the vascular endothelium (Deshpande et al. 1998, Rodrigues & Progulske-Fox 2005, Belanger et al. 2006), apoptosis in endothelial cells (Chiu 1999), platelet aggregation (Herzberg & Weyer 1998, Sharma et al. 2000), induction of macrophage foam cells' formation (Qi et al. 2003) and increased MMP-9 activity (Lee et al. 2006). These mechanisms could explain partially the association between periodontal disease and cardiovascular events by direct action of the microorganism.

The incidence of the genus *Actinomyces* in bacteraemia was previously demonstrated (Messini et al. 1999, Forner et al. 2006a). It has been reported that these microorganisms also produce cervicofacial, thoracic and abdominal actinomycosis (Tastepe et al. 1998, Wagenlehner et al. 2003, Oostman & Smego 2005), intracranial and renal abscesses (Brook 2004, Ewald et al. 2006) and bacterial endocarditis (Mardis & Many 2001, Julian et al. 2005). The high incidence of this genus during bacteraemia in patients with periodontitis and their capability of affecting other remote organs should be carefully considered.

This study evaluated the presence of bacteria in peripheric blood immediately after SRP and 15 and 30 min. after the procedure was finished. The highest incidence was observed immediately after treatment (73.8%) and decreased 30 min. after treatment (19%).

P. gingivalis, *E. corrodens* and *M. micros* were found in the blood 30 min. after procedure. The capability of neutralizing the microorganism in blood varies among patients and may represent an additional risk factor for developing remote infections. Moreover, degradation of Gram-negative bacteria by the immune system can promote the expression of lypopolisaccharides in the peripheric blood initializing cell activation and the subsequent production of pro-inflammatory cytoquines (Ide et al. 2004). SRP can also elevate significantly the levels of proinflammatory cytoquines in a short time (D'Aiuto et al. 2004, 2005, Forner et al. 2006b). More studies are needed to determine the effects of bacteraemia, the degradation of bacteria in plasma and its influence in the elevation of proinflammatory markers.

We found one patient with GAgP with bacteraemia before treatment. A possible explanation could be that the patient did not follow the recommendations, although all factors were controlled to the extent possible. Tooth brushing and chewing have demonstrated induction of bacteraemia in periodontitis patients (Kinane et al. 2005, Forner et al. 2006a).

Bacteria were detected in 80.9% of the patients using culture techniques. *P. gingivalis*, *M. micros* and *E. corrodens* were detected at different times after treatment in the same patient but only in two of them it was possible to isolate the same bacteria during three times after treatment. In some cases, detection of microorganisms was not possible immediately after treatment (T2) or 15 min. after treatment (T3). This result suggests that although blood cultures are considered the gold standard, evaluation of bacteraemia by this method can lead to false-negative results and cannot detect bacteria degraded by the immune system. This indicates the need of evaluating other methods for the detection of periodontopathic microorganisms in bacteraemia studies using molecular or immunologic techniques, especially when investigating the association between periodontal and cardiovascular disease.

Previous studies have demonstrated *P. acnes* after bacteraemia induced by periodontal procedures (Messini et al. 1999, Kinane et al. 2005). In this study, these bacteria were isolated with relative frequency, but considering that this microorganism does not belong to the normal flora of the subgingival plaque, we can presume that its presence may be due to skin contamination after the collection of the blood sample.

This study supports the evidence that bacteraemia is highly associated with periodontopathic microorganisms after SRP in patients with severe periodontitis. The high incidence of periodontopathic microorganisms renders SRP-induced bacteraemia an in vivo model that allows the study of the relationship between periodontal disease associated bacteria and its distant effects in the human body in general, and the interaction between periodontal disease and cardiovascular disease, in particular.

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Clinical Relevance

Scientific rationale for the study: Bacteraemia seems to be critical in the association between periodontal disease and cardiovascular disease. Current information regarding the incidence of periodontopathic microorganisms during bacteraemia in patients with periodontal disease after SRP is limited.

Principal findings: The incidence of periodontopathic microorganisms and other bacteria growing under anaerobic conditions during bacteraemia was high in patients with periodontitis after SRP. *P. gingivalis* and *M. micros* were the periodontopathic microorganisms more frequently isolated from blood during bacteraemia. There was also a high frequency of *Actinomyces* spp among these patients.

Practical implications: Clinical practice guidelines for the management of periodontal disease among patients with a high risk of cardiovascular disease should consider the high incidence of bacteraemia by periodontopathic microorganisms after SRP.