Clinical Periodontal and Microbiologic Parameters in Patients With Acute Myocardial Infarction

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Background: The aim of this study was to evaluate the impact of clinical periodontal parameters and the presence of periodontal pathogens in patients with acute myocardial infarction (AMI).

Methods: A total of 104 subjects (54 patients with AMI and 50 healthy controls) were included. Subgingival plaque samples were analyzed for periodontal pathogens *Aggregatibacter actinomycetemcomitans* (*Aa*; previously *Actinobacillus actinomycetemcomitans*), *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*; previously *T. forsythensis*), and *Prevotella intermedi*a (*Pi*) using dot-blot hybridization.

Results: Patients with AMI had a significantly higher frequency of probing depths (PDs) ≥4 mm than controls (39.2% versus 14.9%; $P < 0.0001$). Among different cutoff levels, the frequency of >50% sites with PDs ≥4 mm showed the highest discrepancy between both groups (33% versus 0%; $P < 0.001$). All periodontal pathogens were overrepresented in patients with AMI and positively correlated with increased periodontal PD and clinical attachment level (CAL). After adjustment for age, gender, smoking, body mass index, hypertension, plaque index, statin intake, and ratio of cholesterol to high-density lipoprotein, *Pg* remained a significant predictor for AMI (odds ratio [OR]: 13.6; 95% confidence interval [CI]: 3.1 to 59.8; $P = 0.0005$). Furthermore, the simultaneous presence of *Aa* + *Pg* ($P = 0.0005$) and *Aa* + *Pg* + *Tf* ($P = 0.0018$) were found with significantly higher frequency in patients with AMI than controls.

Conclusions: The results of our study confirm an association between periodontitis and AMI in which periodontal destruction was correlated with the presence of periodontal pathogens. In particular, *Pg* might be considered a potential risk indicator for AMI. *J Periodontol 2009;80:1581-1589.*

KEY WORDS

Coronary heart disease; infection; myocardial infarction; periodontal diseases; periodontal pathogens; periodontitis.

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Cardiovascular diseases (CVDs) are still among the most frequent causes of death in industrialized countries. Although classical risk factors for atherosclerosis such as hyperlipoproteinemia, hypertension, smoking, and male gender have been established, they only account for a part of cardiovascular risk.[1,2] Accumulating evidence points to an infectious etiology of CVD, suggesting bacterial or viral infections as potential risk factors for the manifestation of endothelial dysfunction and, in consequence, CVD manifestation.[3-5] One of the most prevalent and multibacterial infectious diseases is periodontitis. In the last decades, this disease has been considered a previously undervalued additional risk factor for CVD.[6,7]

Several longitudinal and cross-sectional studies have indicated an association between periodontitis and coronary heart disease (CHD).[8-15] Bacteremia with systemic dissemination of periodontopathic bacteria, lipopolysaccharides (LPSs), and proinflammatory mediators are supposed to account for these associations.[12] However, some studies[16-19] did not confirm such

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associations. There may be various reasons for this inconsistency, such as the use of different diagnostic criteria for periodontitis (e.g., the Community Periodontal Index of Treatment Needs,[13] the Russel index,[16] and probing depth [PD])[9] and different considerations of CHD risk factors. Second, host factors regulating inflammatory immune response, which have been identified in both diseases,[20,21] might influence the interaction between CHD and periodontal disease. Finally, the impact of periodontal pathogens on the relationship between CHD and periodontitis has only been included in recent articles.[11,13-15]

The manifestation of periodontitis is accompanied by opportunistic changes in the subgingival microflora leading to the predominance of Gram-negative bacteria in periodontal pockets. Increased numbers of these pathogens have been found in the pockets of patients with CHD, but they have also been detected in atherosclerotic plaques.[22-25] However, not all studies on atherosclerotic lesions confirmed these results,[26-28] and, moreover, different periodontal bacteria harvested in periodontal lesions have been associated with CHD.[11,13,15] Thus, more information about the role of microbiota in the association of periodontitis and CHD is necessary.

From a practical point of view, the concern must be raised whether there are clinical periodontal thresholds and/or particular periodontal pathogens that are associated with an increased risk for CHD. Therefore, the aim of this study was to investigate clinical periodontal and microbiologic parameters in patients with acute myocardial infarction (AMI). More specifically, we wanted to determine: 1) whether periodontitis is associated with an increased risk for AMI; 2) at what diagnostic periodontal threshold the odds for AMI are highest; and 3) whether the prevalence of periodontal pathogens in subgingival biofilm is increased in patients with AMI compared to control subjects.

MATERIALS AND METHODS

Study Population

The clinical study design and study population have already been described in a preceding article.[29] Briefly, the study protocol was as follows: a case control study of subjects aged 35 to 61 years was developed in cooperation between the Department of Internal Medicine I, Central Hospital Augsburg and the Department of Operative Dentistry and Periodontology, University Hospital Aachen. All subjects were enrolled in the study from August 2006 through August 2007. The study protocol was approved by the Bavarian Ethical Committee, Munich, Germany, and informed written consent was obtained from all patients before their examination.

The test group consisted of 60 patients with AMI who had been referred to the Department of Internal Medicine I of the Central Hospital in Augsburg. The diagnosis of AMI was based on contemporary criteria according to the Guidelines of the European Society of Cardiology and the American College of Cardiology.[30] Accordingly, patients with typical chest pain, a positive troponin test, and a characteristic electrocardiogram (ECG) with either ST segment elevation or non-ST segment depression were considered indicative for AMI. Criteria for exclusion from the study were the presence of diabetes mellitus, pregnancy, pronounced obesity (body mass index [BMI] >35 kg/m^2), and antibiotic therapy <2 months before the investigation. Patients with <14 teeth, and those who underwent a periodontal treatment within the preceding 6 months before the AMI event were also excluded. Periodontal examination of the patients was done within the first 2 months after the proceeding infarction.

The control group consisted of 55 subjects devoid of any history of CVDs and matched for gender and age status. Matching was done in the following way: after complete examination of all patients with AMI, three age groups (35 to 45, 46 to 55, and >55 years) were defined, and for each age group, the same number of controls with the same percentage of males and females were recruited (frequency matching). All control subjects were enrolled from a private dental office in the central hospital neighborhood and randomly selected on the basis of patient card numbers, birth date, and gender. Each of the control subjects was referred to a specialist in cardiology who conducted a comprehensive medical examination including an ECG to state that they were free from CHDs. Exclusion criteria were the same as those of patients with AMI. After exclusion, 54 cases and 50 controls were available for the study.

For evaluation of anamnestic data, all participants were interviewed according to a standardized protocol. Subjects were asked about their medical history, medications, smoking habits, history of periodontitis, and oral hygiene. BMI and serum laboratory parameters were assessed in each subject.

Oral Examination

In all patients and controls, a comprehensive periodontal examination, including the assessment of plaque index (PI),[31] gingival index (GI),[31] periodontal PD, and clinical attachment level (CAL), was performed by one dentist (JC). PD and CAL values were recorded at six sites per tooth. CAL (distance between the cemento-enamel junction and bottom of the pocket) was obtained by adding the PD values to gingival-recession values (distance between the gingival margin and cemento-enamel junction). All measurements were performed with a millimeter-graded, pressure sensitive probe†† set to a probing force of 0.25 N.

†† Hawe Click-Probe, Kerr Hawe, Bioggio, Switzerland.

To obtain acceptable intraexaminer reproducibility for PD and recession values, a calibration exercise was performed 4 weeks prior to the study by duplicate measurements of PD and gingival-recession values of 30 teeth randomly selected. Intraexaminer reliability was assessed by calculating Cohen's κ coefficient. The simple κ value was 0.958 (95% confidence interval [CI]: 0.921 to 0.994), and the weighted κ was 0.972 (95% CI: 0.948 to 0.996), which were considered acceptable.

PD values ≥4 mm were regarded as indicative of periodontal pathology.[32] To analyze at which threshold the discrepancy of increased pathologic PDs between groups was the highest, different cutoff levels were tested for the number of sites with PD ≥4 mm. Furthermore, the diagnosis of periodontitis was defined as previously described[33,34] by the percentage of sites with clinical attachment loss >3 mm: 1% to 32% = mild; 33% to 66% = moderate; and 67% to 100% = severe.

Identification of Periodontal Pathogens

For identification of the periodontal pathogens *Aggregatibacter actinomycetemcomitans* (*Aa*; previously *Actinobacillus actinomycetemcomitans*), *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*; previously *T. forsythensis*), and *Prevotella intermedia* (*Pi*), subgingival microbial samples were obtained from all patients and controls. The deepest pocket of each arch quadrant was selected for sampling. After supragingival debridement, one sterile paper point‡‡ was inserted to the bottom of each selected pocket for 15 seconds. All four paper points with subgingival plaque samples were pooled together into a transfer tube and stored with a temperature of –25°C for further laboratory analysis.

The detection of the periodontal pathogens was performed with dot-blot hybridization using oligonucleotide probes derived from 16S rRNA and labeled with digoxigenin-11-deoxyuridine 5-triphosphate. The DNA probes§§ were developed according to Conrads et al.[35] and optimized in a computerized comparison against 12,000 bacterial 16S rRNA/DNA sequences and tested empirically against various bacterial strains and the human genome. They proved to be >99.99% specific for *Aa*, *Tf*, *Pg*, and *Pi*, respectively. Nucleic acids were isolated by the aid of a kit‖‖ or simple boiling. Hybridization was performed following the instructions of the manufacturer¶¶ and standard procedures.[36] All laboratory analyses were performed in a masked manner.

According to the manufacturer, the dot-blot hybridization assay has a detection limit of 10^2 to 10^3 cells and is highly specific, reacting negatively with up to 10^9 oral competitor cells. With the digoxigenin-11-dTUP labeling## and chemiluminescent substrate detection system,*** a dynamic range of six magnitudes (10^3 to 10^8) with a linear cell-to-signal ratio was achieved.

Statistical Analyses

Primary outcome values of continuous variables were computed as the mean and standard deviation. The unpaired *t* test was used to assess differences for theses variables between the AMI and control groups. For data of categoric variables, absolute and relative frequencies were calculated and compared between both groups using the Fisher exact test.

Moreover, the influence of all potential periodontal and microbiologic risk factors (variables) on AMI was calculated in multivariable logistic regression analyses. Adjustment was done for the potential confounders age, gender, BMI, smoking, PI, history of hypertension, statin intake, and ratio of cholesterol to high-density lipoprotein (HDL). The statistical significance of each variable was assessed by the *P* value of the respective Wald χ^2 test. Odds ratios (ORs) were calculated according to Woolf[37] and given with their 95% CIs. In cases with quasi-complete separation, ORs and corresponding CIs were calculated using the penalized maximum-likelihood estimation according to Firth.[38]

To avoid spurious significance among multiple inferences (type-one error), the Bonferroni adjustment was used to interpret the significance of *P* values. Therefore, *P* values <0.0019 (n tests = 27) were regarded as statistically significant test results. Data processing and all statistical analyses were performed using a statistical software packages.†††,‡‡‡

RESULTS

Descriptive and Laboratory Results

Descriptive data for the AMI and control groups are presented in Table 1. Age, gender, and BMI did not differ between groups. Cases had significantly more smokers and a higher number of mean pack-years than controls. In the AMI group, there were more individuals with hypertension. Blood lipids (low-density lipoprotein [LDL], HDL, triglycerides, and total cholesterol) were decreased in cases compared to controls.

Table 2 displays the type of medicaments used by patients with AMI and controls. Cases took more antihypertensives than controls, which corresponded to the increased number of subjects with hypertension in the AMI group. Similarly, in accordance with increased blood lipids, the intake of statins was higher in patients with AMI than controls. Furthermore, in

‡‡ Roeko, Langenau, Germany.
§§ LCL Biokey, Aachen, Germany.
‖‖ QIAamp Blood & Tissue Kit, Qiagen, Hilden, Germany.
¶¶ LCL Biokey.
Roche Diagnostics, Mannheim, Germany.
*** Roche Diagnostics.
††† SAS, Version 9.1, SAS Institute, Cary, NC.
‡‡‡ Microsoft Office Excel SAS, Version 11.1.1, Microsoft, Redmond, WA.

Table 1.

Descriptive and Laboratory Parameters

Variable	Patients With AMI (n = 54)	Controls (n = 50)
Age (years; mean [SD])	50.8 (6.3)	51.7 (6.5)
Age range (years)	35 to 60	36 to 61
Females (n [%])	4 (7.4)	3 (6.0)
Smoking habit (n [%])	28 (51.9)	11 (22.0)
Pack-years (mean [SD])	24.8 (25.8)	11.8 (17.1)
BMI (kg/m^2; mean [SD])	27.1 (3.6)	27.2 (3.7)
History of hypertension (n [%])	34 (63.0)	17 (34.0)
LDL (mg/dl; mean [SD])	118.2 (41.7)	147.1 (34.6)
HDL (mg/dl; mean [SD])	43.5 (16.0)	50.0 (12.7)
Triglycerides (mg/dl; mean [SD])	160.4 (99.0)	175.1 (100.5)
Cholesterol (mg/dl; mean [SD])	197.0 (44.8)	224.7 (38.1)

Table 2.

Medicaments Used in Patients With AMI and Controls

Type of Medicament	Patients With AMI (n [%])	Controls (n [%])
Anti-hypertensives	29 (53.7)	6 (12.0)
Beta blockers	25 (46.3)	4 (8.0)
ACE inhibitors	11 (20.4)	1 (2.0)
Anti-platelet and anticoagulant agents	24 (44.4)	1 (2.0)*
ASS	22 (40.7)	1 (2.0)*
Clodiprogel	12 (22.2)	0 (0.0)
Coumarin derivatives	1 (1.9)	0 (0.0)
Thyroid drugs	4 (7.4)	6 (12.0)
Statins	19 (35.2)	4 (8.0)

ACE = angiotensin-converting enzyme; ASS = acetylsalicylic acid.
* ASS medication because of arrhythmia (atrial fibrillation).

contrast to control subjects almost every second AMI patient took anti-platelet medicaments.

Periodontal Examination

Cases with AMI had slightly fewer teeth than controls. For all periodontal parameters assessed, the test group showed worse results than the control group. Cases had a significantly higher frequency of moderate and severe periodontitis than controls (P <0.0011). Mean periodontal PD and mean clinical attachment loss were significantly increased among cases (P <0.0002). Moreover, the number of sites with PD ≥4 and ≥6 mm was significantly elevated in the test group. Hygiene indexes (PI and GI) were also significantly increased among cases with AMI. After adjustment for age, gender, BMI, hypertension, statin intake, smoking, and total cholesterol to HDL, comparisons remained statistically significant for GI, mean PD, mean CAL, and percentage of PDs ≥4 mm (Table 3).

The extent of periodontitis was assessed as the proportion of sites with PD ≥4 mm that exceeded 10%, 20%, 30%, 40%, 50%, and 60% of sites, respectively. The mean proportional distributions of cases and controls for the six different cutoff levels are presented in Figure 1. At all cutoff levels, significant differences were found. The greatest difference between cases and controls based on the highest OR was observed at the 50% cutoff level (33.3% versus 0%, respectively; P <0.0001; OR: 51.2; 95% CI: 2.99 to 877.20). However, statistical significance according to the Bonferroni significance level was lost after adjustment for potential confounders (OR: 14.4; 95% CI: 1.5 to 2,097.7; P= 0.017).

Microbiologic Results

All periodontal pathogens were found with increased frequency among cases compared to controls. In univariable analysis, *Aa* and *Pg* showed statistically significant differences between both groups. After adjustment for potential confounders including PI and the Bonferroni correction, only *Pg* remained significantly associated with AMI (Table 4).

To test whether associations to single periodontal bacteria are based on the simultaneous occurrence of more than one periodontal pathogen, the frequencies of different combinations of the bacteria were compared between both groups. All combinations between two of the four investigated pathogens were found with significantly higher frequency among cases with AMI. After adjustment for the potential confounders, the combined occurrence of *Aa* and *Pg* showed the highest OR for AMI followed by the combined presence of *Tf* and *Pg* and *Pg* and *Pi* and the presence of three bacteria, *Aa*, *Pg*, and *Tf*. According to the Bonferroni correction, only the adjusted P values of the combinations *Aa* + *Pg* and *Aa* + *Tf* + *Pg* remained statistically significant (Table 5).

Furthermore, significantly positive correlations were found among all four bacteria. In particular, *Aa* showed a positive relationship to the three other bacteria. *Pg* was positively correlated to *Tf*. Also, the presence of *Aa*, *Tf*, and *Pi* was positively correlated with increased periodontal PDs and clinical attachment loss (Table 6).

Table 3.

Periodontal Parameters in Patients With AMI and Controls

Variable	Patients With AMI (n = 54)	Controls (n = 50)	*P* Value (crude)	*P* Value (adjusted)*
Missing teeth (n; mean [SD])	4.4 (3.5)	3.9 (3.8)	0.4241	0.0970
PI (mean [SD])	1.06 (0.52)	0.60 (0.39)	<0.0001†	0.0055
GI (mean [SD])	0.94 (0.46)	0.46 (0.30)	<0.0001†	0.0002†
PD (mm; mean [SD])	4.6 (0.9)	3.7 (0.8)	<0.0001†	0.0002†
CAL (mm; mean [SD])	5.4 (1.2)	4.5 (1.1)	0.0003†	0.0012†
% sites with PD ≥4 mm (mean [SD])	39.2 (20.2)	14.9 (13.9)	<0.0001†	0.0001†
% sites with PD ≥6 mm (mean [SD])	7.2 (8.3)	2.9 (5.4)	0.0061	0.0485
% sites with CAL ≥4 mm (mean [SD])	52.2 (23.6)	32.6 (22.6)	0.0002	0.0027
% sites with CAL ≥6 mm (mean [SD])	13.4 (15.3)	8.1 (12.2)	0.0569	0.1553
Moderate + severe periodontitis (n [%])	41 (75.9)	22 (44.0)	0.0011†	0.0077
Mild periodontitis (n [%])	13 (24.1)	28 (56.0)	0.0011†	0.0077

* Adjusted for age, gender, BMI, hypertension, statin intake, smoking, and total ratio of cholesterol to HDL.
† Statistically significant according to the Bonferroni correction (*P* <0.0019).

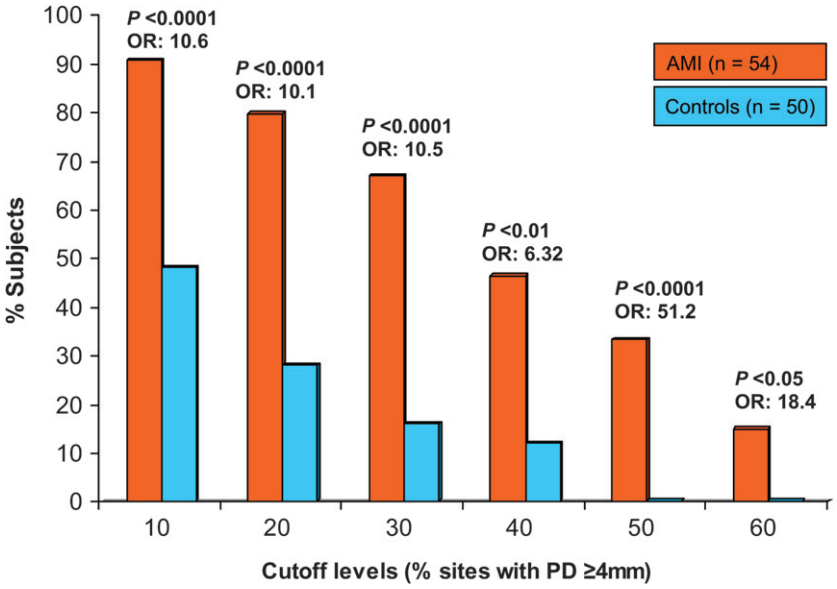

Figure 1.
Percentage of subjects with PD ≥4 mm at different proportions of sites (cutoff levels) assessed in patients with AMI and controls. P values based on the Fisher exact test. ORs were calculated according to Woolf[37] or Firth,[38] respectively.

DISCUSSION

Before discussing the results of our study, some aspects of validity will be addressed. The study was performed in a case-control design. Although cases with AMI were recruited from the hospital, control subjects were enrolled from a private practice, which may suggest a kind of selection bias. However, controls were derived from the same geographic area and belonged to the same community as cases. Also, controls were not selected on the basis of any dental or periodontal diagnosis. Thus, although bias due to this aspect cannot completely be excluded, its impact may be limited in this study.

As to the selection criteria for the study participants, exclusion of subjects with diabetes mellitus and matching for gender, age, and ethnic descent resulted in a minimum number of confounders. Nevertheless, matching for smoking was not achieved as the number of smokers was limited among control subjects. This resulted in a higher percentage of smokers among patients with AMI. Together with age and gender, smoking is known to be a risk factor for periodontitis and CVD.[39,40] To control for this aspect, smoking, age, and gender were included as confounders in multivariable models and compared to the univariable results.

Regarding cardiovascular risk factors, a history of hypertension was higher in cases than controls. Hypertension is an important risk factor for CHD[1,2] and, therefore, has been included in multivariable models. The decreased level of blood lipids in cases corresponds to previous observations[41] and, moreover, might be based on the high percentage of statin medication in this group. Therefore, blood lipids (ratio of cholesterol to HDL) and statin intake were considered potential confounders for adjusted analyses.

The presented hygiene indexes, which were increased in cases compared to controls, must be interpreted with caution. Because a high percentage of patients with AMI received anti-platelet drugs (Table 2), which can increase gingival bleeding measurements, the value of the GI as a periodontal diagnostic

Table 4.

Frequency of Detection of Periodontal Pathogens in Patients With AMI and Controls

Variable	Patients With AMI (n [%])	Controls (n [%])	Unadjusted Analysis		Adjusted Analysis*	
			OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Aa	44 (81.5)	15 (30.0)	10.3 (4.1 to 5.6)	<0.0001†	6.5 (1.6 to 26.7)	0.0089
Tf	40 (74.1)	25 (50.0)	2.9 (1.3 to 6.5)	0.0124	2.6 (0.7 to 9.9)	0.1612
Pg	42 (77.8)	13 (26.0)	10.0 (4.0 to 24.5)	<0.0001†	13.6 (3.1 to 59.8)	0.0005†
Pi	42 (77.8)	34 (68.0)	1.6 (0.7 to 3.9)	0.2634	3.1 (0.7 to 14.5)	0.1460

* Adjusted for age, gender, BMI, hypertension, statin intake, smoking, PI, and total ratio of cholesterol to HDL.
† Statistically significant according to the Bonferroni correction (*P* <0.0019).

Table 5.

Frequency of Detection of Combinations of Periodontal Pathogens in Patients With AMI and Controls

Variable	Patients With AMI (n [%])	Controls (n [%])	Unadjusted Analysis		Adjusted Analysis*	
			OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Aa + *Tf*	37 (68.52)	9 (18.00)	3.7 (2.1 to 6.7)	<0.0001†	2.7 (1.2 to 6.4)	0.0198
Aa + *Pg*	36 (66.67)	5 (10.00)	7.1 (3.5 to 14.5)	<0.0001†	6.8 (2.3 to 19.9)	0.0005†
Aa + *Pi*	36 (66.67)	12 (24.00)	3.8 (1.9 to 6.9)	<0.0001†	3.0 (1.2 to 7.6)	0.0164
Tf + *Pg*	33 (61.11)	8 (16.00)	4.3 (2.3 to 8.0)	<0.0001†	5.1 (1.7 to 15.1)	0.0030
Tf + *Pi*	32 (59.26)	18 (36.00)	2.2 (1.2 to 3.8)	0.0149	2.6 (1.0 to 6.9)	0.0613
Pg + *Pi*	33 (61.11)	10 (20.00)	4.1 (2.1 to 8.2)	<0.0001†	4.3 (1.7 to 10.9)	0.0026
Aa + *Tf* + *Pg*	31 (57.41)	3 (6.00)	3.8 (2.3 to 6.2)	<0.0001†	3.5 (1.6 to 7.8)	0.0018†

* Adjusted for age, gender, BMI, hypertension, statin intake, smoking, PI, and total ratio of cholesterol to HDL.
† Statistically significant according to the Bonferroni correction (*P* <0.0019).

criterion is limited. Further, patients with AMI could be expected to show worse PI scores due in part to their stay in the intensive care unit and longer hospital stay. Nonetheless, the (temporarily) increased PI might have favored conditions for the colonization of periopathogens, which is why PI was included as a potential confounder in the multivariable analyses of periodontal pathogens in cases versus controls.

The clinical periodontal findings of our study point to a positive association of (moderate and severe) periodontitis and AMI, which has been reported in most papers.[8-11,13,15] A few authors[16] did not find an association between periodontitis and CHDs. In the latter, however, periodontitis was diagnosed without differentiating the severity and extent of periodontitis. In contrast, in our AMI group, mean periodontal PD, mean clinical attachment loss, percentage of sites with pathologic PDs (PD ≥4 mm), and the number of deep pockets (PD ≥6 mm) were significantly higher than among controls. Consequently, we can state a higher prevalence and increased severity of periodontal disease in patients with AMI, which was in accordance with the results of other studies.[9,15] This issue might be of importance because higher numbers and increased depths of periodontal pockets favor the colonization of pathogens resulting in a higher risk for bacteremia.[17] Moreover, multivariable analysis showed the same deviations; e.g., potential risk factors such as smoking might confound AMI and periodontitis but could not explain the worse periodontal status in patients with AMI. To define a threshold for periodontitis, at which the odds for AMI was greatest, different cutoff levels with pathologic PDs were compared between both groups. The presence of >50% of sites with PD ≥4 mm showed the highest discrepancy between groups. Although this parameter lost its significance after adjustment for known risk factors, it seemed to be of potentially clinical relevance. Renvert et al.[10] reported a similar result based on radiographs: The cutoff value of "50% of sites with ≥4 mm" of approximal bone loss was the best predictor for AMI. In our study, radiographs were not available, and periodontitis was defined with clinical measurements. Though reproducibility might be better in radiographic diagnostics, periodontal bone loss in radiographs can be detected in patients with untreated periodontitis, as well as patients with a history of periodontitis (i.e., radiographic parameters do not necessarily correlate to clinical parameters). Therefore, we regarded sites with PDs ≥4 mm as more suitable surrogates for bacterial infection, which might have systemic effects due to bacteremia.

In the present study, periodontal pathogens were overrepresented in patients with AMI. In univariable

Table 6.

Correlations Between Proven Bacteria and Periodontal Parameters in Patients With AMI*

	Aa	*Tf*	*Pg*	*Pi*
Aa	1.00	0.51†	0.42†	0.53†
Tf	0.51†	1.00	0.44†	0.30‡
Pg	0.42†	0.44†	1.00	0.33‡
Pi	0.53†	0.30‡	0.33‡	1.00
PD	0.54†	0.42†	0.28‡	0.46†
CAL	0.43†	0.35‡	0.17	0.38†

* Spearman correlation coefficient.
† $P < 0.0001$.
‡ $P < 0.05$.

analyses, all examined target bacterial species were found with significantly increased frequency in the AMI group. After adjustment for smoking, age, gender, history of hypertension, statin intake, PI, and ratio of cholesterol to HDL, the association for *Pg* remained statistically significant. Further, combination analyses revealed that the presence of *Pg* and the simultaneous occurrence of *Pg* and *Aa* were significantly associated with an increased risk for AMI (OR: 6.8; 95% CI: 2.3 to 19.9). A less strong but also significant association was found for the combined presence of *Aa*, *Pg*, and *Tf*. The latter findings may be based on significantly positive correlations between *Aa* and *Tf* as well as *Pg* and *Tf* (Table 6). All periodontopathogenic bacteria were positively correlated with periodontal PDs and CAL underlining the close etiologic relationship between microbes and periodontal destruction in patients with AMI.

In contrast to our results, Nonnenmacher et al.[15] found only *Pi* to be associated with coronary artery disease. In another study on patients with CHD, only an increased number of *Aa* and total bacterial load were evident.[13] Renvert et al.[14] reported a higher bacterial load of *Pg*, *Tf*, and *Trepohema denticola (Td)*, but not *Aa*, in patients with AMI. All of these studies had comparable study designs. There might be several reasons for these inconsistent findings: In contrast to our study, antibiotic therapy in the last (at least 2) months before the study examination and previous periodontal treatment had not been reported as exclusion criteria in the other studies. However, antibiotic therapy and periodontal treatment can reduce the number of relevant periodontal pathogens beyond the detection limit and introduce a bias. Furthermore, different techniques and thresholds for the detection of periodontal pathogens might also contribute to the differences. After all, also the periodontal diagnosis may in part explain the discrepant results. Acute[14] and chronic[13,15] forms of CHDs might differ as to periodontal microbiologic findings. This was reflected by Sakurai et al.,[42] who found *Aa* in oral samples in 33% of patients with acute coronary syndrome (ACS; n = 15), whereas no *Aa* (0 of 13) was found in chronic CHD patients ($P < 0.05$). Sakurai et al.[42] also reported higher serum titers of immunoglobulin G against *Aa* in ACS patients compared to patients with chronic CHD.

The particular role of *Aa* and *Pg* for CVDs has already been suggested by other authors. Both pathogens are known to possess a high periodontal pathogenicity,[43] and they are able to invade and persist in periodontal tissues.[44] Chronic inflammatory disintegration of the periodontal epithelial barrier promotes the dissemination of periodontal bacteria through the bloodstream into peripheral endothelia. As emphasized, not all studies investigating periodontal pathogens in atheromatous plaques had positive results,[27,28] and, moreover, a study[26] investigating both the presence of particular bacteria in periodontal pockets and atheromatous plaques did not necessarily find corresponding outcomes. However, positive results were frequently reported for *Aa*[22-24,45] and *Pg*.[22,23,45] Further, Pussinen et al.[46] found a combined antibody response against *Aa* and *Pg* to be directly associated with CHD and inversely with HDL-cholesterol concentration. They suggested that periodontitis may impair reverse cholesterol transport favoring the development of atherosclerotic lesions. An alternate explanation for the associated bacteria discussed in the present study might include the role of LPSs produced by Gram-negative bacteria as the dominating periodontopathogens. LPS is considered a major systemic inflammatory burden for chronically infected patients because it enhances the expression of cytokines[47]and CD40 ligands on platelets,[48] which might contribute to proatherogenic effects either.

It must be noted that the microbiologic findings of our study are based on a cross-sectional investigation. Thus, no causal relationship between periodontal pathogens and AMI can be concluded. Direct involvement of bacteria in pathogenesis of CVDs,[46] indirect effects due to antibacterial immune response,[49] and the potential influence of endotoxins[48] can be assumed but not proved. Nevertheless, our results revealed a significantly increased prevalence of periodontal pathogens in patients who had AMI which supports the hypothesis of *Pg* particular role in CVDs.

CONCLUSIONS

Within the limits of the present study, the following main conclusions can be drawn: 1) the results demonstrate a worse periodontal status in patients with AMI compared to healthy controls; 2) among different

cutoff values, the presence of >50% sites with PDs ≥4 mm showed the highest discrepancy between patients with AMI and control subjects; and 3) together with known confounders, *Pg* might be a potential risk indicator for future AMI.

We emphasize that the data of this article must be interpreted with necessary caution. The number of cases and controls were relatively small, and only a few of the results remained significant after correction for multiple comparisons or adjustment for confounders. Because of the case-control design, this study can generate hypotheses but cannot prove them. Nonetheless, our findings might contribute to approaches in risk evaluation and prevention strategies in CHDs.

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