Results From the Periodontitis and Vascular Events (PAVE) Study: A Pilot Multicentered, Randomized, Controlled Trial to Study Effects of Periodontal Therapy in a Secondary Prevention Model of Cardiovascular Disease

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Abstract

Background—In the Periodontitis and Vascular Events (PAVE) pilot study, periodontal therapy was provided as an intervention in a secondary cardiac event prevention model through five coordinated cardiac– dental centers.

Methods—Subjects were randomized to either community care or protocol provided scaling and root planing to evaluate effects on periodontal status and systemic levels of high-sensitivity C-reactive protein (hs-CRP).

Results—After 6 months, there was a significant reduction in mean probing depth and extent of 4-or 5-mm pockets. However, there were no significant differences in attachment levels, bleeding upon probing, or extent of subgingival calculus comparing subjects assigned to protocol therapy (n = 151) to those assigned to community care (n = 152). Using intent-to-treat analyses, there was no significant effect on serum hs-CRP levels at 6 months. However, 48% of the subjects randomized to community care received preventive or periodontal treatments. Secondary analyses demonstrated that consideration of any preventive or periodontal care (i.e., any treatment) compared to no treatment showed a significant reduction in the percentage of people with elevated hs-CRP (values >3 mg/l)

at 6 months. However, obesity nullified the periodontal treatment effects on hs-CRP reduction. The adjusted odds ratio for hs-CRP levels >3 mg/l at 6 months for any treatment versus no treatment among non-obese individuals was 0.26 (95% confidence interval: 0.09 to 0.72), adjusting for smoking, marital status, and gender.

Conclusion—This pilot study demonstrated the critical role of considering obesity as well as rigorous preventive and periodontal care in trials designed to reduce cardiovascular risk.

Keywords

C-reactive protein; cardiovascular diseases; controlled clinical trial; obesity; periodontitis; prevention

Over the last decade, there have been numerous case-control and longitudinal studies that generally, but not unanimously, support a statistically significant association between the clinical presentation of periodontal disease and more severe cardiovascular disease (CVD). Linkages between periodontitis and various CVD diagnostic outcomes include the presence of coronary heart disease, peripheral arterial disease, myocardial infarctions, severe atherosclerosis (thick carotid intimal-medial wall thickness as determined by ultrasound and also with echo-indicated calcification),[1–4] acute coronary syndrome,[5] and non-hemorrhagic stroke.[6] These associations, adjusted for a variety of cardiovascular risk factors, appear to be mild to moderate in magnitude with increased odds ratios (ORs), relative risks, and hazards ratios in studies showing a positive association ranging from 1.2 to 3.9.[7,8] Although not all populations or studies show statistically significant associations, the majority of the studies, as summarized by meta-analyses, show significant associations even after adjusting for traditional risk factors including smoking, blood lipids, race, gender and obesity.[7,8] Whether these associations are causally related or due to underlying genetic or behavioral risk factors that are common to both conditions remains unknown.

It has been suggested that clinical periodontal-disease measurements that are used to quantify the level of exposure in these studies provide only a unidimensional estimate of the burden of periodontal disease and may not fully represent the infectious and inflammatory stress associated with periodontal disease.[9] Recent studies[10,11] of CVD association, using measurements that capture clinical signs and periodontal infectious and inflammatory measurements as exposures, generally show stronger associations than just clinical parameters of disease. This point is important to understand the association between the two conditions, as it is now generally believed that atherogenesis and plaque rupture, two critical elements of cardiovascular pathogenesis that lead to chronic disease burden and clinical events, are a consequence of systemic and vascular inflammatory processes.

Inflammation impairs the function of the endothelium, promotes atheroma formation within the major elastic arteries, and compromises the structural integrity of the arterial plaque by creating vascular regions of unstable plaque that lead to susceptibility to thrombotic and embolic events.[12] Thus, the traditional role of lipid abnormalities in cardiovascular risk, such as an increase in blood levels of low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein, comprise one causal pathway leading to cardiovascular events, and it is now clear that inflammation poses a second pathogenic pathway.[12] For example, it is widely cited that about half of all cardiovascular events occur among subjects who do not have any of the traditional Framingham risk factors[12] (e.g., lipid abnormalities including high cholesterol and low LDL). It is in this context that systemic inflammation is thought to promote atherogenesis and plaque instability, a process that can be reflected by increased levels of serum biomarkers of inflammation, such as C-reactive protein (CRP), soluble intracellular adhesion molecule, or interleukin (IL)-6. An infection of oral origin can represent one potential source

of systemic infectious and inflammatory stress that might contribute to the morbidity and mortality of CVD.

Despite the fact that many studies have shown associations that link periodontal disease to CVD, there have been relatively few studies to address the potential effects of periodontal treatment on surrogate markers of cardiovascular risk or cardiovascular outcomes. Recent studies have suggested that periodontal treatments can reduce levels of serum high-sensitivity (hs)-CRP (as determined by hs-CRP assay), lower IL-6,[13–15] and improve endothelial function as measured by flow-mediated dilation.[15–18]

It is not known whether the vascular lesions that are associated with periodontal disease are fully reversible. Indeed, not all multifactorial diseases can be reversed by removing an etiologic component. This is particularly relevant for chronic diseases in which the causative agent was present, perhaps insidiously, for decades prior to clinical onset.

Thus, to better understand whether periodontal disease was a potentially modifiable cause of CVD, the Periodontitis and Vascular Events (PAVE) pilot study was designed as a randomized controlled intervention trial to determine whether we could: 1) recruit patients with CVD into a periodontal treatment study;2)provide periodontal treatment in a combined university/ community setting and measure improvements in periodontal health; 3) determine whether periodontal treatment affects the levels of serumhs-CRP;and4) follow-up with subjects to show that we could evaluate cardiovascular endpoints.

In the following report of the PAVE pilot randomized controlled trial (RCT), we demonstrate that periodontal therapy improved periodontal health but did not reduce the level of hs-CRP compared to the community care control group using an intent-to-treat analysis. However, we performed secondary analyses to further examine the importance of community care, the response to therapy, and other risk modifiers on study outcomes. The findings from this pilot study may provide important insight into how to optimally design future studies aimed at providing periodontal care to reduce adverse cardiovascular outcomes and lower levels of surrogate biomarkers of cardiovascular risk.

MATERIALS AND METHODS

Study Design

In two previous publications,[19,20] we described the study aims, the design, patient recruitment, patient demographics, treatments received by both protocol provided and community care groups, subject retention, clinical periodontal responses to treatment, and adverse event outcomes. As described in Couper et al.,[19] "the specific aims of the PAVE pilot study were: 1) to organize field centers and the administrative infrastructure necessary to perform a definitive clinical trial of periodontal treatment in the secondary prevention of CVD; 2) to use these field centers and the clinical trial administrative infrastructure to design and implement a pilot RCT with the primary goal of testing the efficacy of the infrastructure in recruiting and enrolling patients, and in data management; and 3) to obtain information from the pilot trial on feasibility, including data on compliance, periodontal and cardiovascular outcomes, and adverse events." In this article, we report the effects of periodontal therapy on changes in clinical status (probing depth [PD], bleeding on probing [BOP], and attachment level) and the biomarker data (serum hs-CRP and the periodontal gingival crevicular fluid [GCF]-IL-1β levels). Our hypotheses were that periodontal therapy would reduce the levels of PD, BOP, and attachment loss (AL) and lower hs-CRP and GCF-IL-1β levels among the protocol treatment group compared to the community care group.

Patient Population and Treatments

The study, conducted from January 2003 to June 2005, was approved by human subject committees at all five field centers (University at Buffalo, University of North Carolina at Chapel Hill, Boston University, Oregon Health and Science University, and University of Maryland), and written informed consent was obtained prior to subject enrollment. Oversight of the study was provided by a Data Safety Monitoring Board (DSMB) appointed by the National Institute of Dental and Craniofacial Research(NIDCR), National Institutes of Health (NIH), Bethesda, Maryland, that included both periodontal and cardiology experts. The study randomized 303 subjects (beginning in March 2003; enrollment ended in December 2004, with the last follow-up visits in June 2005) with periodontal disease and a previous history of recent cardiovascular events. For the cardiovascular criteria, participants had to have ≥50% blockage of one coronary artery or, within 3 to 36 months prior to enrollment, have had a coronary event, including myocardial infarction, coronary artery bypass graft surgery, or coronary transluminal angioplasty with or without a stent. The periodontal inclusion criteria were the presence of at least six natural teeth (not including hopeless teeth), including third molars, with at least three teeth having PD≥4 mm, at least two teeth with interproximal AL ≥2 mm, and with ≥10% of sites having BOP. Once subjects were identified as eligible, hopeless teeth were extracted, and subjects were randomized to a either a community care group, consisting of oral hygiene instruction plus a letter of referral to seek periodontal care, or a protocol "intensive treatment" group consisting of oral-hygiene instruction plus scaling and root planing under anesthesia using apiezoelectric ultrasonic scaler,[##] suturing as needed. All therapists received standardized instruction and training in the use of the piezoelectric scaler. Full-mouth periodontal examinations were conducted at baseline and at 6 and 12 months collecting data on the plaque index,[21] gingival index,[22] calculus index,[23] PD, AL, and BOP using trained and calibrated examiners. Serum and GCF were collected at all three visits.[23,24]

Laboratory Analyses

GCF measurements of IL-1β were performed measuring at four GCF sampling sites per subject.[24] Briefly, immunoreagents[***] were used to create standard curves using a an ELISA plate reader[†††] in a range of 0 to 250 pg/ml with a lower sensitivity of 1.5 pg/ml. The coefficient of variance (CV) for standard curves was in the rangeof8%to12%, which was within our laboratory operational maximum of a CV <15% for this assay.

Serum hs-CRP concentrations were measured by latex-enhanced nephelometry.[‡‡‡] The hs-CRP assay uses a monoclonal antibody attached to polystyrene particles and fixed-time kinetic nephelometric measurements. This fully automated system creates a seven-point standard curve from 0.4975 mg/ml (1:40 dilution of Rheumatolgy standard) to 0.0078 mg/ml (1:2,560 dilution). The nephelometer makes a 1:400 dilution to measure sample CRP concentrations between 3.5 and 210 mg/l and a 1:20 dilution <3.5 mg/l. This is the only hs-CRP assay that has been approved by the United States Food and Drug Administration (FDA) for use in assessing the risk of cardiovascular and peripheral vascular disease.

Data Analyses

Baseline characteristics of the protocol treatment and community care groups were expressed as the prevalence for categoric data and differences in proportions by the χ^2 test. Continuous variables were expressed as means, and standard errors were computed. Differences in means were tested for statistical significance using *t* tests. Differences in response variables at 6 and

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[##]Cavitron, Dentsply, York, PA.
[***]Cayman ELISA Kit, Cayman Chemical, Ann Arbor, MI.
[†††]Spectro-Max M2, Molecular Devices, Sunnyvale, CA.
[‡‡‡]BN high-sensitivity CRP assay on a BN II nephelometer, Dade Behring (Siemens), Deerfield, IL.

12 months for community versus protocol treatment were assessed by analysis of covariance (ANCOVA) adjusting for baseline values for each patient. *P* values <0.05 were considered statistically significant.

Three study design issues were identified a priori as potential modifiers of the effects of the planned experimental intervention: 1) non-protocol preventive and periodontal treatments provided to subjects randomized to the community care group; 2) subject obesity that serves to increase hs-CRP levels irrespective of periodontal disease status;[23] and 3) hs-CRP levels within the normal range at baseline may be difficult to reduce further, and improvements may only be evident in patients with elevated levels at baseline or in preventing an increase in hs-CRP from normal to high during the period of study.

As secondary analyses, we stratified subjects based upon dental-use patterns, obesity, and hs-CRP values. Dental-use patterns were assessed by an administered questionnaire during visits and follow-up telephone inquiries. Utilizations were categorized by preventive (prophylaxis only) or scaling and root planing plus prophylaxis. To explore the effects of community treatment on outcomes, the community referral group was stratified into those who reported receiving any periodontal care (any treatment, i.e., either prophylaxis, scaling and root planing, or gum surgery) or no periodontal treatment. Three subjects had periodontal surgery following scaling and root planing with prophylaxis and were pooled with the scaling and root planing group. Subjects were also stratified by obesity using a body mass index (BMI) value >30 for obesity.

Additional analyses were performed using a three-level categoric definition of hs-CRP as described by Ridker[25] with hs-CRP ranges 0 to <1 mg/l = low or normal, 1 to 3 mg/l = intermediate, and >3 mg/l = high. hs-CRP at values >3 mg/l approximates the highest decile as described by Ridker,[25] and a high hs-CRP value reflects a "high risk" category for future cardiovascular events. Differences in the proportions of subjects with high hs-CRP among various treatment and obesity groups were analyzed by the χ^2 test. Bivariate associations were determined for the relationship between baseline characteristics and elevated hs-CRP. Logistic regression models were developed for high hs-CRP using the subset of the community care group who indicated that they did not receive any preventive or periodontal treatment as the referent population (no treatment). Baseline characteristics were included in the adjusted model for hs-CRP if they were significantly (*P* <0.05) related to elevated hs-CRP. Separate logistic models were computed stratifying by obesity to compute odds ratios (OR) and 95% confidence intervals (CI) for high hs-CRP.

RESULTS

The baseline demographics of the community care and protocol treatment groups appear in Table 1. The baseline characteristics for both groups have similar distributions of race, gender, hypertension, diabetes, smoking history, BMI, mean age, level of education, marital status, employment, and type of CVD event that qualified them for the study. There were a significantly greater number of subjects on cholesterol-lowering drugs in the community care group with 96.7% of subjects (147 of 152) taking these medications compared to 88.7% of subjects in the protocol treatment group. Further analyses of this difference showed that this variation in medication-usage patterns was not a significant modifier of any of the periodontal or inflammatory outcomes in this study, and no post hoc adjustments were indicated.

In Table 2, the baseline periodontal measurements for the community care and the protocol treatment groups show that they were well matched in terms of disease severity at baseline as indicated by measures of extent PD ≥4 mm, mean PD, mean AL, and extent BOP. Plaque indices and gingival indices were also similar for both groups (data not shown). The meanGCF-

IL-1β levels were not significantly different at baseline comparing the two groups. Similarly, the baseline mean serum hs-CRP levels and the percentage of subjects with high hs-CRP were not different. Equal distribution of baseline characteristics between the two comparison groups suggested that randomization was effective.

The effects of periodontal therapy are shown at 6months in Table 3 and at 1 year in Table 4. The treatment protocol had a significant effect on improving periodontal status at 6 months compared to community care (Table 3). The *P* values are statistically significant for extent PD ≥4 mm ($P = 0.001$) and mean PD($P = 0.0009$)without significant effects on mean AL(borderline at *P*= 0.052) or extent BOP scores ($P = 0.16$)adjusting for baseline values for each patient using ANCOVA. Overall, there was an improvement in pocket reduction and a trend for slightly lower bleeding scores and less AL at 6 months comparing the protocol intervention group to the community care group. The GCF-IL-1β levels showed no significant differences between groups at 6months. The effects of periodontal intervention on serum hs-CRP show that there were no effects of protocol therapy on serum hs-CRP levels (Table 3) at 6months.

Compared to baseline, both groups showed a trend toward higher values at 6 months, but there were no significant differences in mean CRP values at 6 months adjusting for baseline values for each subject by ANCOVA. Thus, using intent-to-treat principles, the intervention resulted in an improvement in periodontal health but did not result in a significant change in GCF-IL-1β or serum hs-CRP levels at 6 months. As shown in Table 4, the clinical improvement seen among those treated under protocol was not significantly different from baseline at the 1-year follow-up. At the 1-year visit, GCF and serum data were available for 37 subjects. Table 4 demonstrates that, at 1 year, there were no significant differences, using ANCOVA, in any of the clinical parameters or biomarker values comparing the community care group to the protocol treatment group after adjusting for baseline values, except for the extent subgingival calculus scores (values ≥2), which were significantly lower among those subjects receiving protocol-mandated care ($P = 0.009$). Furthermore, the clinical status for these 37 subjects was not significantly different from baseline at 1 year, except for lower subgingival calculus scores. However, because relatively few subjects were seen at 1 year, detailed secondary analyses were limited to the baseline and 6-month data.

Secondary Analyses of hs-CRP

One design issue of the trial that concerned us from the beginning was that subjects assigned to the community care group might receive periodontal care during the study period, an intervention that could potentially diminish any possible differences between the two study groups. At the onset before randomization, we also extracted hopeless teeth and provided home care instructions, thereby instituting some level of infection control among those who were ultimately assigned to the community care group. Furthermore, subjects who were identified as having periodontal disease and were randomized to the community care group were also provided with referral letters to seek additional care.

Table 5 shows the dental care–use patterns for all study participants. Overall, 48% of the community care control group received one or more preventive or periodontal therapies during the 6-month study period, including prophylaxis, scaling and root planing, and periodontal surgery. Some of this information regarding scaling and root planing was previously published. [19] In Table 5, those subjects assigned to the community care group received significantly higher levels of non-study-care than those receiving therapy on protocol, including total number of dental visits, numbers of prophylaxis, and subgingival scaling and root planing. These use differences suggest that perhaps many who received care as part of the PAVE protocol chose to substitute study-provided care for normal community care dental visits, although they were advised to continue to seek routine dental care during the study. The protocol treatment provided a net change (SD) of −0.30 (0.45) mm (6-month baseline value) which was more

improvement than that seen among the community care group (−0.13 [0.34] mm; *P* = 0.001). However, both groups demonstrated a significant improvement in PD at 6 months relative to baseline (Tables 2 and 3). These findings suggested that secondary analyses to explore the effects of periodontal care provided to subjects randomized to the community care group appeared warranted.

As secondary analyses, subjects were regrouped into those who received any periodontal treatment and those who received no treatment, irrespective of group assignment. Also, subjects were stratified based upon obesity. The community no treatment group was defined as those subjects assigned to the community care group who did not report having any periodontal care during the study period (6 months; n = 79). The community care group was further subdivided into those that received a prophylaxis only or a prophylaxis plus scaling and root planing. Three individuals in the community care group received periodontal surgery and were combined into the community scaling and root planing group. The any treatment group (n = 224) included protocol treatment plus those in the community care group that had either prophylaxis, scaling and root planing, or periodontal surgery. In Figure 2, the percentage of subjects with high hs-CRP (hs-CRP >3mg/l) are shown for obese and non-obese individuals at 6 months for the community care group, stratified upon type of care provided within the community setting, the protocol treatment group, and the pooled any treatment group. There were two striking findings. First, there was no difference in the proportion of subjects who have elevated hs-CRP irrespective of treatment among those 73 obese individuals (BMI >30). It appears that periodontal treatment of obese individuals with high hs-CRP had no effect in the prevalence of elevated hs-CRP levels at 6months. Secondly, there was a marked lower proportion of subjects with hs-CRP ≥3 mg/l at 6 months among those non-obese subjects who received either a community prophylaxis or scaling and root planing, whether assigned to community care or protocol. Among non-obese individuals in the community who received no treatment, 43.5% had high hs-CRP at 6 months. In contrast, there was a strikingly lower proportion of subjects with high hs-CRP among those subjects who received a prophylaxis (6.25%; *P* = 0.009), scaling and root planing (8.3%; *P* = 0.03), and protocol therapy (22.2%; *P* = 0.04). It is noteworthy that these two community treatment groups also demonstrated clinical improvements at 6 months relative to baseline. For example, the mean (SE) PD change in millimeters for the four groups was: no treatment (−0.07 [0.06]), prophylaxis (−1.17 [0.06]), scaling and root planing (−0.20 [0.08]), and protocol treatment (−0.30 [0.03]). Combining all treated, non-obese subjects into the any treatment group showed, with statistical significance, a lower proportion of subjects with high hs-CRP from43.5% in the no treatment group to 17.6% (*P* = 0.006) in the any treatment group.

Logistic regression models were used to compute the ORs shown in Table 6 for having high hs-CRP (>3 mg/l) at 6 months based upon periodontal treatment provided either in the community setting or on protocol compared to the community no treatment group as the referent population stratifying by obesity or using all treated subjects. As illustrated in Figure 2, there were no significant findings regarding the risk for high hs-CRP among obese individuals irrespective of treatment category. However, any treatment among all subjects resulted in a statistically significant 2.38-fold lower odds for high hs-CRP (OR = 0.42 [95% CI = 0.18 to 0.995]). The effects were even stronger among the non-obese subjects, with a 3.85-fold lower odds for having high hs-CRP at 6 months with any treatment (OR = 0.26 [95% CI = 0.09 to 0.72]). The community-provided prophylaxis showed a significant reduction in OR among non-obese subjects, whereas the lower OR (0.09) seen with community scaling and root planing was not statistically significant among non-obese individuals. The protocol treatment had no effect among the obese individuals but significantly lowered the OR to 0.34 (95% CI = 0.12 to 0.98) among the non-obese subjects, representing a statistically significant 2.9-fold reduction in the number of subjects with high hs-CRP at 6 months compared to those non-treated individuals in the community setting.

DISCUSSION

General Comments Regarding Study Design and Implementation

This pilot study resulted in several important operational and outcome assessment insights. During the first year of the trial, the PAVE enrollment was inadequate because the periodontal disease levels for eligibility were initially too stringent, and the logistic engagement of cardiovascular-qualified potential subjects into the dental component of the investigation was not convenient for patients. Periodontal disease criteria were lessened, and we learned that dental screening must be performed in cardiology settings rather than as a two-step referral process. In the PAVE trial, the sample size was not designed to be adequate to test the hypothesis that periodontal treatment reduces the incidence of secondary cardiovascular events, but it was adequately powered to test the hypothesis that periodontal treatment may improve periodontal status, lower GCF-IL-1β, and reduce the serum level of CRP at 6 months post-treatment compared to the community care group. Although relatively few subjects were available for analysis at 1 year due to slow initial enrollment, several significant trends can be seen at 1 year that warrant discussion. In addition, the preliminary findings provide further guidance for future study design.

Effects of Periodontal Therapy on Periodontal Status and GCF-IL-1β Levels

The treatment protocol resulted in a significant improvement in periodontal status at 6 months as assessed by reduction of PD, but there was little or no change in attachment levels, BOP, or subgingival calculus. This unexpected result suggested that a more rigorous protocol for treatment may be necessary in a population of cardiovascular patients. The effects of treatment were attenuated at 1 year. This finding is consistent for treatments provided in a community setting in which close patient follow-up including oral hygiene and supportive maintenance is not provided at intervals that are more frequent than 6 months. Subjects were initially identified as cardiac patients who were asked to participate in a dental trial and were not initially patients seeking periodontal care, which may in part explain the relatively modest response to periodontal therapy. Furthermore, many patients who were randomized to the therapy arm apparently skipped community dental appointments, as their non-study dental use was significantly lower (specifically, 57% of the rate) than the group randomized to community care. This finding points to the need in future trials to provide a more stringent maintenance program to maintain a more stable improvement in periodontal status in this secondary cardiac prevention model.

Although there were not significant differences in GCF-IL-1β as a result of periodontal therapy at 6 months, GCF-IL-1β responses to scaling and root planing are usually short term, occurring shortly after treatment (i.e., 4 to 6 weeks) and are highly correlated to clinical changes.[27] Thus, the reestablishment of periodontal pockets and bleeding scores at 6 months would be expected to result in a reestablishment of GCF-IL-1β levels. The proportion of the community care group (48%) that received some form of preventive or periodontal care and demonstrated clinical improvement was an unexpected outcome. In our previous studies on subjects who were in medical studies but enrolled in secondary dental studies (e.g., the Piedmont Study[28] or the Oral Conditions and Pregnancy Study[29]), subjects showed much lower rates of dental care–seeking behaviors when informed of their periodontal disease, closer to a range of 12% to 15% (unpublished data). This may be a reflection of this particular patient population who are more health care motivated due to their recent cardiovascular event or perhaps a more general appreciation for the potential linkages between oral health and CVD than in the population at large. It is important to note that many patients had some level of infection control prior to randomization, and this alone may have prompted many patients to seek additional community care. Irrespective of the reasons, the effects of community care are significant because they resulted in clinical improvements and a reduction in systemic inflammation among those

individuals who have high hs-CRP. This finding points to an important design issue for future studies in that the level of community care provided to control subjects, both preventive and periodontal therapy, needs to be considered. In a community-based trial, treatments provided by private practitioners cannot be suspended, and ethically, patients who are newly diagnosed as having periodontal disease must be informed and advised to seek care. Thus, the level of periodontal care received by patients within the community remains a wild-card variable that can be monitored but not readily controlled in community-based studies and is likely to affect all intent-to-treat analyses.

Effects of Periodontal Therapy on Serum hs-CRP

From an intent-to-treat perspective, the pilot intervention therapy did not result in a significant reduction in mean hs-CRP or a reduction in the proportions of individuals with high hs-CRP (>3 mg/l) compared to the community care group. Several previous cross-sectional studies[23,25,30] indicated that periodontal disease was associated with elevated serum levels of hs-CRP. Although serum CRP elevation is a nonspecific marker of the acute phase response that can be elicited by a broad range of stimuli, elevated hs-CRP has been demonstrated to be predictive of CVD events including myocardial infarction and stroke.[25] Furthermore, several studies[13,14,31] that include periodontal treatment demonstrated a lowering of the serum CRP level following periodontal treatment, but not all studies show reductions in systemic inflammation following periodontal care.[32] This evidence suggested that periodontal treatment may reduce the levels of serum CRP in certain circumstances and, therefore, could potentially be evaluated as an intermediate surrogate endpoint in a pilot intervention trial that would be underpowered to demonstrate an effect on cardiovascular events. A study by Slade et al.[24] demonstrated that both periodontal disease and obesity were associated with increased serum hs-CRP levels and that there was a significant interaction between obesity and periodontal disease on the serum hs-CRP level. The cross-sectional study[24] could imply that treating periodontal disease among people with obesity might have no effect on lowering the serum hs-CRP level unless the patient also lost weight at the same time. In the PAVE study, obesity was associated with higher levels of hs-CRP as reflected in the baseline differences in hs-CRP levels comparing obese and non-obese subjects (3.81 [2.99 SD] versus 1.91 [2.07 SD] mg/l, respectively; P <0.0001). Furthermore, in the PAVE study, the hs-CRP was non-responsive to the effects of periodontal therapy among obese individuals, confirming the fact that obesity overwhelms the mild elevation of hs-CRP that is elicited by periodontal disease. This is potentially due to the well-established concept that adipose tissue is both a fat-storage organ as well as a source of inflammatory adipokines that serve to increase serum markers of inflammation, including hs-CRP.[12] Similar concerns were possible for other factors that could potentially modify serum hs-CRP levels, including concomitant infections or inflammatory conditions, gender, smoking, and diabetes. Adjusting for these confounders in our intent-to-treat study design, we found a non-significant overall treatment effect of lowering the prevalence of elevated CRP at 6 months (>3 μg/l; OR = 0.55 [95%CI = 0.24 to 1.24]). Thus, whereas the effect of periodontal treatment on lowering serum CRP may be demonstrated in a well-controlled clinical trial, it was not clear whether periodontal treatment could have an impact on hs-CRP levels in a community setting because periodontal disease is just one of the potential stressors that can activate the acute phase response. We would suggest that, in clinical trials conducted in university settings, study designs select subjects with more severe periodontal disease and higher serum hs-CRP values upon randomization, and the level of care provided to controls is minimal in order to maximize the potential difference between treated and untreated groups. Indeed, the results from the PAVE study suggest that even a single prophylaxis preventative therapy visit could have an effect on lowering the prevalence of high hs-CRP (Fig. 2) at 6 months in non-obese individuals. Furthermore, in the PAVE study, the proportion of non-obese individuals with high hs-CRP was lower at 6 months, irrespective of the treatment provided.

The PAVE findings on the effects of treatment on hs-CRP need to be interpreted with caution because they are based on secondary analyses. Nonetheless, several important trends seem to be emerging regarding the effects of periodontal therapy on hs-CRP that may be important for future study design.

First, control subjects must be truly untreated individuals, because even minimal care such as prophylaxis alone appears to have an impact on reducing high hs-CRP. Secondly, the effects of treatment are not uniform across the spectrum of hs-CRP values. Typically, hs-CRP values within the population are not normally distributed and are often categorized into three levels: hs-CRP values between 0 and <1mg/l are categorized as normal; values from 1 to 3 mg/l are intermediate; and values >3 mg/l are high. Hs-CRP values above 10 mg/l are suggestive of acute infection or stress that would be transient and not reflective of the chronic level. Therefore, patients with levels >10 mg/l are advised to have repeat values within a week to allow the transient peak to subside to chronic homeostatic levels.[33] In some ways, periodontal disease appears to operate within the range of 1 to 10 mg/l and can contribute to drift in hs-CRP within this range depending upon biofilm load, periodontal status, and periodontal disease activity. Close examination of the data on the effects of treatment suggest that there is little change in the chronic steady-state level of hs-CRP following care. The effect of periodontal therapy appears to prevent any increase in hs-CRP to levels >3 mg/l, a trend that is common among patients with periodontal disease. Non-obese patients without periodontal disease or other infectious or inflammatory challenges will typically have hs-CRP levels <1 mg/l and remain in the normal range. Patients with periodontal disease have hs-CRP values that are higher (with values, on average, of 2.3 mg/l per 10% increase in extent PD ≥4 measurements[23]) and, upon repeat measurements, show time-dependent variations. Thus, preventive and periodontal treatments appear to lower hs-CRP levels below 3 mg/l and also prevent the upward drift of hs-CRP above 3 mg/l that occurs among subjects with untreated periodontal disease. It appears likely that the upward shifts in hs-CRP levels seen among untreated periodontitis patients may be mediated by infectious or inflammatory events occurring within the periodontium, but that remains to be proven. It is interesting to note that, at 1 year, the OR for high hs-CRP is 0.54 (95% CI = 0.10 to 2.78) for any treatment compared to the community no treatment group, adjusting for BMI. This is not statistically significant, but the trend suggests that the effects of treatment on hs-CRP may potentially persist >6 months.

CONCLUSIONS

Collectively, these data suggest that periodontal therapy may lower hs-CRP levels among non-obese cardiovascular patients if the levels are >3mg/l initially, and it may prevent a drift to levels >3mg/l for those that are in the intermediate range of 1 to 3mg/l. It does not appear that periodontal therapy diminishes hs-CRP levels among individuals with hs-CRP levels <3 mg/l in this patient population. The chronic burden of CVD as an inflammatory process may alone serve to keep a mild elevation of hs-CRP present, irrespective of the added burden of periodontal disease. Obesity acts to increase hs-CRP levels and to possibly nullify any periodontal treatment effects on hs-CRP levels. These data indicate that, overall, there were significant improvements in periodontal clinical health at 6 months following the periodontal intervention compared to community care. In our previous publications of this study,[19,34] we demonstrated that cardiovascular patients can be successfully recruited through cardiology practices and successfully randomized and that cardiovascular events can be monitored for 1 year post-therapy and endpoints adjudicated. Thus, this pilot study establishes feasibility and important insight for designing future interventional trials.

Acknowledgments

References

1. DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM. Dental disease and risk of coronary heart disease and mortality. BMJ 1993;306:688–691. [PubMed: 8471920]

2. Beck JD, Offenbacher S. Systemic effects of periodontitis: Epidemiology of periodontal disease and cardiovascular disease. J Periodontol 2005;76(11 Suppl):2089–2100. [PubMed: 16277581]

3. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: The atherosclerosis risk in communities (ARIC) study. Arterioscler Thromb Vasc Biol 2001;21:1816–1822. [PubMed: 11701471]

4. Nakib SA, Pankow JS, Beck JD, et al. Periodontitis and coronary artery calcification: The atherosclerosis risk in communities (ARIC) study. J Periodontol 2004;75:505–510. [PubMed: 15152812]

5. Czerniuk MR, Górska R, Filipiak KJ, Opolski G. Inflammatory response to acute coronary syndrome in patients with coexistent periodontal disease. J Periodontol 2004;75:1020–1026. [PubMed: 15341362]

6. Elter JR, Offenbacher S, Toole JF, Beck JD. Relationship of periodontal disease and edentulism to stroke/TIA. J Dent Res 2003;82:998–1001. [PubMed: 14630902]

7. Bahekar AA, Singh S, Saha S, Molnar J, Arora R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: A meta-analysis. Am Heart J 2007;154:830–837. [PubMed: 17967586]

8. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: A systematic review and meta-analysis. J Periodontol 2007;78:2289–2302. [PubMed: 18052701]

9. Beck JD, Offenbacher S. Relationships among clinical measures of periodontal disease and their associations with systemic markers. Ann Periodontol 2002;7:79–89. [PubMed: 16013220]

10. Beck JD, Eke P, Lin D, et al. Associations between IgG antibody to oral organisms and carotid intima-medial thickness in community-dwelling adults. Atherosclerosis 2005;183:342–348. [PubMed: 15893320]

11. Desvarieux M, Demmer RT, Rundek T, et al. Relationship between periodontal disease, tooth loss, and carotid artery plaque. The oral infections and vascular disease epidemiology study (INVEST). Stroke 2003;34:2120–2125. [PubMed: 12893951]

12. Hansson GK, Robertson AK, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol 2006;1:297–329. [PubMed: 18039117]

13. D'Aiuto F, Parkar M, Andreou G, et al. Periodontitis and systemic inflammation: Control of the local infection is associated with a reduction in serum inflammatory markers. J Dent Res 2004;83:156–160. [PubMed: 14742655]

14. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J Dent Res 2005;84:269–273. [PubMed: 15723869]

15. Elter JR, Hinderliter AL, Offenbacher S, et al. The effects of periodontal therapy on vascular endothelial function: A pilot trial. Am Heart J 2006;151:47.e1–47.e6. [PubMed: 16368290]

16. Mercanoglu F, Oflaz H, Öz O, et al. Endothelial dysfunction in patients with chronic periodontitis and its improvement after initial periodontal therapy. J Periodontol 2004;75:1694–1700. [PubMed: 15732873]

17. Seinost G, Wimmer G, Skerget M, et al. Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. Am Heart J 2005;149:1050–1054. [PubMed: 15976787]

18. Tonetti MS, D'Aiuto F, Nibali L, et al. Treatment of periodontitis and endothelial function. N Engl J Med 2007;356:911–920. [PubMed: 17329698]

19. Couper DJ, Beck JD, Falkner KL, et al. The periodontitis and vascular events (PAVE) pilot study: Recruitment, retention and community care controls. J Periodontol 2008;79:80–89. [PubMed: 18166096]

20. Beck JD, Couper DJ, Falkner KL, et al. The Periodontitis and Vascular Events (PAVE) pilot study: Adverse events. J Periodontol 2008;79:90–96. [PubMed: 18166097]

21. Silness J, Löe H. Periodontal disease in pregnancy II: Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–135. [PubMed: 14158464]

22. Löe H, Silness J. Periodontal disease in pregnancy I: Prevalence and severity. Acta Odontol Scand 1963;21:533–551. [PubMed: 14121956]

23. Machtei EE, Christersson LA, Zambon JJ, et al. Alternative methods for screening periodontal disease in adults. J Clin Periodontol 1993;20:81–87. [PubMed: 8436636]

24. Slade GD, Ghezzi EM, Heiss G, Beck JD, Riche E, Offenbacher S. Relationship between periodontal disease and C-reactive protein among adults in the atherosclerosis risk in communities study. Arch Intern Med 2003;163:1172–1179. [PubMed: 12767953]

25. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363. [PubMed: 12551853]

26. Zhong Y, Slade GD, Beck JD, Offenbacher S. Gingival crevicular fluid interleukin-1beta, prostaglandin E2 and periodontal status in a community population. J Clin Periodontol 2007;34:285–293. [PubMed: 17378884]

27. Beck JD, Koch GG. Characteristics of older adults experiencing periodontal attachment loss as gingival recession or probing depth. J Periodontal Res 1994;29:290–298. [PubMed: 7932023]

28. Beck JD, Sharp T, Gary GK, Offenbacher S. A 5-year study of attachment loss and tooth loss in community-dwelling older adults. J Periodontal Res 2006;32:516–523. [PubMed: 9379319]

29. Lieff S, Boggess KA, Murtha AP, et al. The Oral Conditions and Pregnancy study: Periodontal status of a cohort of pregnant women. J Periodontol 2004;75:116–126. [PubMed: 15025223]

30. Elter JR, Champagne CM, Offenbacher S, Beck JD. Relationship of periodontal disease and tooth loss to prevalence of coronary heart disease. J Periodontol 2004;75:782–790. [PubMed: 15295942]

31. Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S. Potential for gingival crevice fluid measures as predictors of risk for periodontal diseases. Periodontol 2000 2003;31:167–180. [PubMed: 12657001]

32. Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. Clin Exp Immunol 1997;107:347–352. [PubMed: 9030874]

33. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PME, Van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol 2000;71:1528–1534. [PubMed: 11063384]

34. Mattila K, Vesanen M, Valtonen V, et al. Effect of treating periodontitis on C-reactive protein levels: A pilot study. BMC Infect Dis 2002;2:30. [PubMed: 12475397]
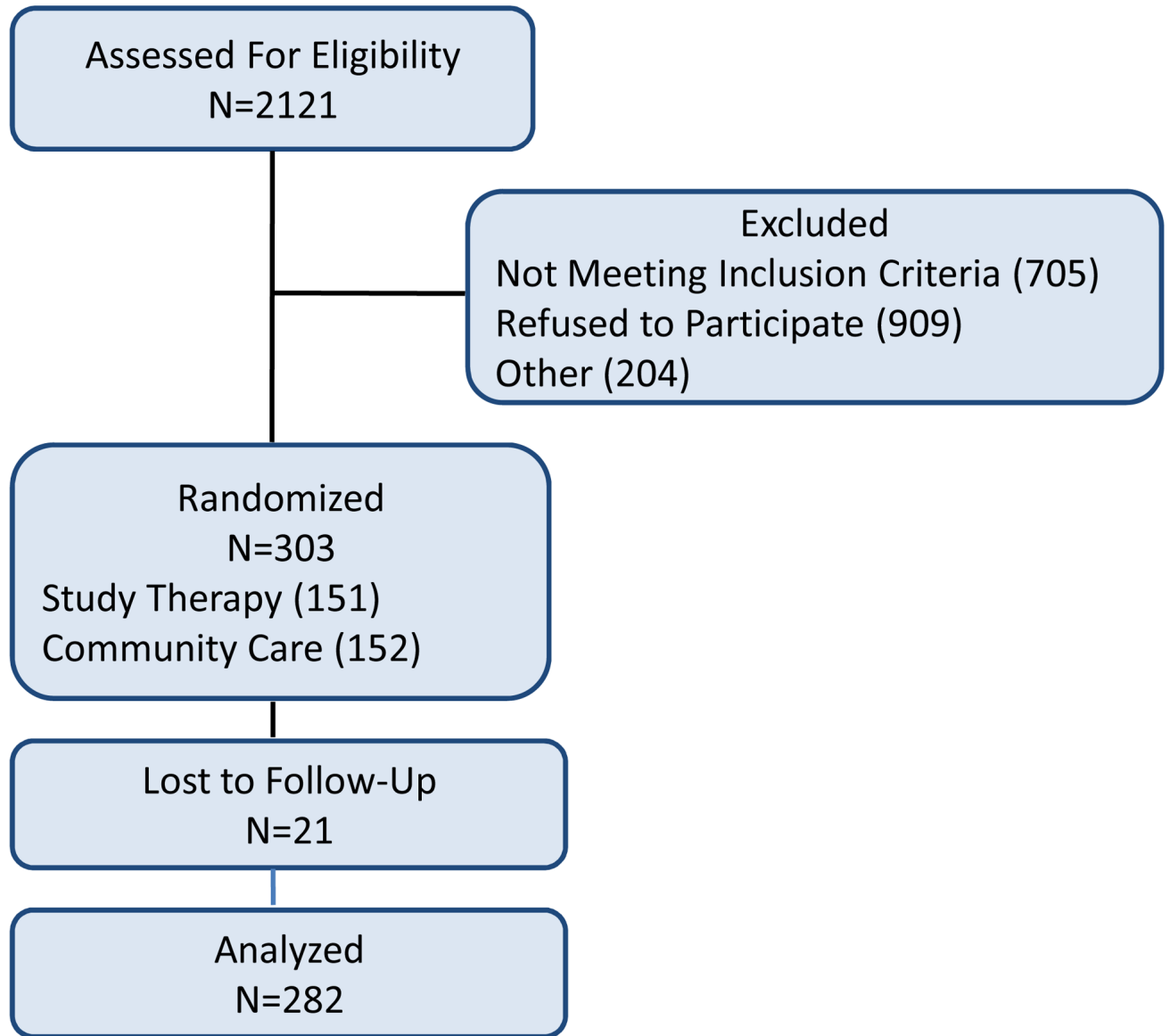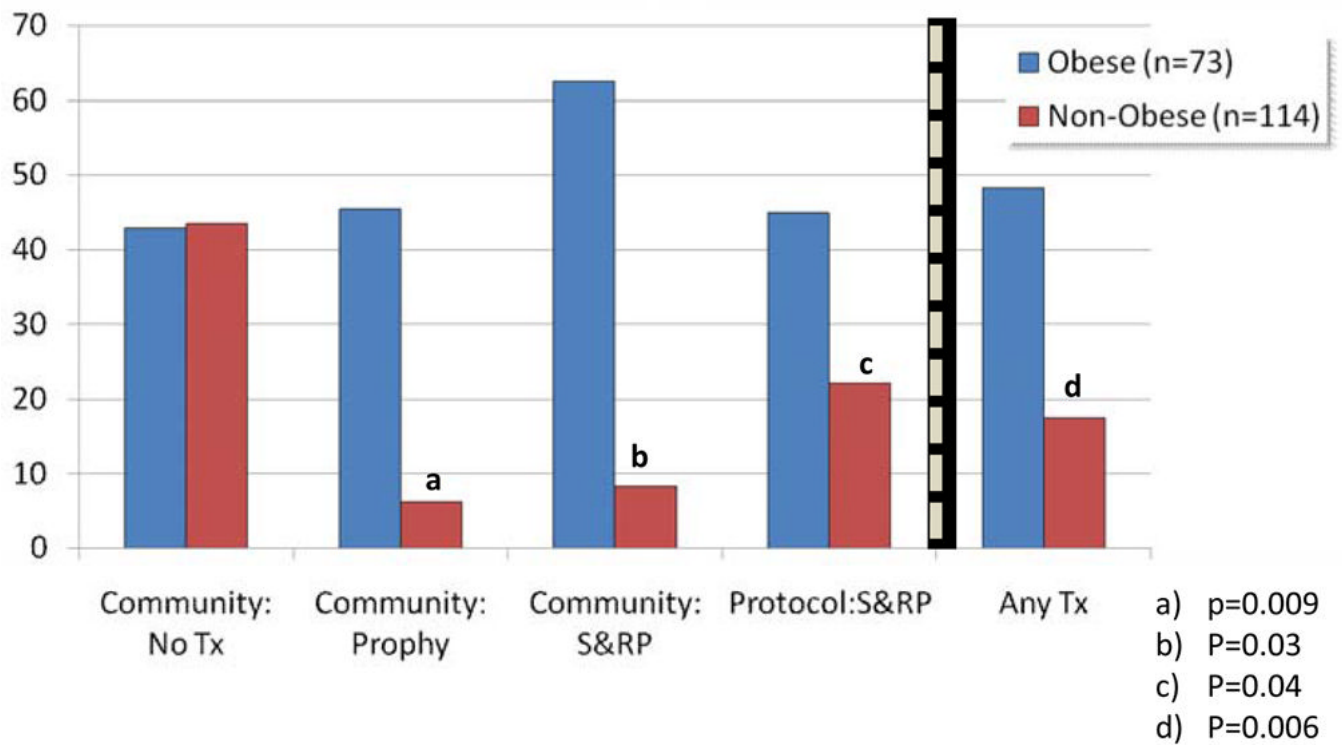
Figure 1.
PAVE study summary.

Figure 2.
Percent of subjects with hs-CRP >3 mg/l at 6 months by treatment group and stratified by obesity. *P = 0.009; †P = 0.03; ‡P = 0.04; §P = 0.006. Tx = treatment; Prophy = prophylaxis; SRP = scaling and root planing. Vertical dashed line designates that "Any Tx" is a composite of the three treatment groups.

Table 1

Demographic Characteristics at Baseline by Group Assignment: The PAVE Study

Demographic Characteristics[*]	Community Control (n = 152)	Protocol Treatment (n = 151)	P Value
Race			
White	127 (49.6)	129 (50.4)	
Non-white	25 (53.2)	22 (46.8)	0.65
Gender			
Male	112 (51.9)	104 (48.2)	
Female	40 (46.0)	47 (54.0)	0.35
Hypertension			
Yes	106 (51.5)	100 (48.5)	
No	46 (48.9)	48 (51.1)	0.69
Diabetes type II			
Yes	31 (50.8)	30 (49.2)	
No	118 (50.4)	116 (49.6)	0.96
Smoking			
Current	22 (43.1)	29 (56.9)	
Former	87 (54.4)	73 (45.6)	
Never	42 (46.2)	49 (53.9)	0.26
Height/weight/BMI (mean [SD])	30.4 (5.36)	30.4 (5.81)	0.94
Age (mean [SD])	59.8 (8.70)	59.5 (9.14)	0.75
Education			
High school or less	62 (50.4)	61 (49.6)	
Greater than high school	90 (50.0)	90 (50.0)	0.94
Married			
Yes	99 (48.5)	105 (51.5)	
No	53 (53.5)	46 (46.5)	0.41
Employed			
Yes	83 (55.7)	66 (44.3)	
No	69 (44.8)	85 (55.2)	0.06
Type of event that made patient eligible (yes)[†]			
Blockage ≥50%	127 (51.2)	121 (48.8)	0.51
MI	56 (46.7)	64 (53.3)	0.30
Angioplasty (with or without stent)	98 (49.3)	101 (50.8)	0.66
CABG	42 (53.9)	36 (46.2)	0.47
Medications (yes)[†]			
Antihypertensive	132 (51.4)	125 (48.6)	0.32
Cholesterol lowering	147 (52.3)	134 (47.7)	0.008
Infection	9 (45.0)	11 (55.0)	0.63
Pain/anti-inflammatory	36 (48.0)	39 (52.0)	0.67

CABG = coronary artery bypass graft.

[*] Values reflect n (%) unless otherwise indicated.

[†] Some participants reported more than one problem or medication.

Table 2

Baseline Periodontal and Serum hs-CRP Measures by Treatment Group

Variable[*]	Community Control	Protocol Treatment	P Value[†]
Extent PD ≥4 mm	20.8 (1.33)	19.9 (1.38)	0.69
Mean PD	2.72 (0.05)	2.69 (0.06)	0.74
N PD ≥5 mm	11.4 (1.05)	11.8 (1.50)	0.83
Extent AL ≥3 mm	51.9 (2.12)	49.6 (2.15)	0.45
Mean AL	2.95 (0.09)	2.79 (0.08)	0.19
Extent BOP	47.3 (2.18)	48.0 (3.13)	0.83
Mean GCF-IL-1**β**	194.4 (129.8)	201.0 (133.8)	0.12
Serum hs-CRP	3.18 (0.36)	3.17 (0.39)	0.76
High CRP (>3 mg/l) (n [%])	48 (33.8)	42 (30.0)	0.98

[*] Values reflect means (SE) unless otherwise indicated.

[†] P values were computed using Student t or χ^2 tests.

IL-1β levels are pg/ml; serum hs-CRP levels are mg/l.

Table 3

Six-Month Follow-Up of Periodontal Assessments, GCF-IL-1β Levels, and Serum Measures of hs-CRP by Treatment Group

Variable[*]	Community Control (n = 102)	Protocol Treatment (n = 126)	P Value[†]
Extent PD ≥4 mm	16.8 (1.56)	13.3 (1.18)	0.001
Mean PD	2.57 (0.07)	2.41 (0.06)	0.0009
N PD ≥5 mm	10.4 (1.36)	7.35 (1.10)	0.0003
Extent AL ≥3 mm	40.7 (2.71)	42.5 (2.22)	0.02
Mean AL	2.72 (0.11)	2.52 (0.08)	0.052
Extent BOP	42.5 (2.56)	38.3 (2.17)	0.16
Extent subgingival calculus	58.6 (3.95)	42.8 (3.14)	0.002
GCF-IL-1**β**	222.3 (22.9)	237.5 (35.0)	0.97
Serum hs-CRP	3.53 (0.45)	3.12 (0.38)	0.97
High CRP (>3 mg/l) (n [%])	31 (34.8)	33 (31.4)	0.62

[*] Values reflect means (SE) unless otherwise indicated.

[†] P values reflect ANCOVA adjusting for baseline values.

IL-1β levels are pg/ml; serum hs-CRP levels are mg/l.

Six-month follow-up data were only available from 228 subjects.

Table 4

One-Year Follow-Up of Periodontal Assessments, GCF-IL-1β Levels, and Serum Measures of hs-CRP by Treatment Group

Variable[*]	Community Control (n = 12)	Protocol Treatment (n = 25)	P Value[†]
Extent PD ≥4 mm	19.5 (6.12)	14.3 (3.51)	0.76
Mean PD	2.77 (0.22)	2.49 (0.16)	0.55
N PD ≥5 mm	9.08 (4.72)	9.48 (3.30)	0.89
Extent AL ≥3 mm	46.8 (8.10)	33.9 (4.94)	0.11
Mean AL	2.95 (0.46)	2.25 (0.19)	0.20
Extent BOP	45.8 (9.06)	37.09 (5.00)	0.88
Extent subgingival calculus	63.8 (12.10)	29.2 (6.06)	0.009
GCF-IL-1**β**	202.6 (102.20)	163.8 (42.30)	0.74
Serum hs-CRP	2.79 (0.71)	3.41 (0.78)	0.74
High CRP (>3 mg/l) (n [%])	5 (33.3)	8 (34.8)	0.93

[*] Values reflect means (SE) unless otherwise indicated.

[†] P values reflect ANCOVA adjusting for baseline values.

IL-1β levels are pg/ml; serum hs-CRP levels are mg/l.

Complete results from 1-year follow-up were only available for 37 subjects.

Table 5

Dental Use During First 6 Months of the Study

	Protocol Treatment	Community Control	*P* Value
Number reporting non-study dental visits (%)	54 (36.5)	93 (64.1)	<0.0001
Number reporting non-study prophylaxis (%)	6 (4.1)	54 (37.2)	<0.0001
Number reporting non-study subgingival scaling and root planing (%)	3 (2.0)	26 (19.9)	<0.0001

Table 6

OR (95% CI) for hs-CRP >3 mg/l at 6 Months

Group	Non-Obese[*]	Obese[*]	All Subjects[†]
Community: no treatment	Referent	Referent	Referent
Community: prophylaxis	0.9 (0.01 to 0.79)[‡]	1.36 (0.4 to 7.88)	0.34 (0.09 to 1.31)
Community: scaling and root planing	0.10 (0.10 to 1.01)	2.55 (0.34 to 19.14)	0.70 (0.19 to 2.56)
Protocol: treatment	0.34 (0.12 to 0.98)[‡]	0.84 (0.22 to 3.24)	0.39 (0.153 to 0.999)[‡]
Any treatment	0.26 (0.09 to 0.72)[‡]	1.05 (0.30 to 3.75)	0.42 (0.181 to 0.995)[‡]

Baseline characteristics were considered for entering into the adjusted logistic models for elevated hs-CRP (data not shown). Significant baseline characteristics included smoking, marital status, and BMI. Gender was also included because of its known relationship[24] to hs-CRP even though it did not reach statistical significance in this dataset ($P = 0.08$).

[*] Adjusted for smoking, marital status, and gender.

[†] Adjusted for smoking, marital status, gender, and BMI.

[‡] Statistically significant.