Clinical and Microbiological Characteristics of Treated Periodontitis Patients on Maintenance Care*


A population of 98 adults previously treated for moderate to advanced adult periodontitis and currently maintained in a recall program for at least one year were recruited for this study. The ultimate purpose of the study is to determine whether the presence of Actinobacillus actinomyctemcomitans, Bacteroides gingivalis, and Bacteroides intermedia in selected periodontal sites can predict the future clinical course of these patients, particularly with respect to disease recurrence. This report presents the clinical design of the study which allows comparisons between sites positive and negative for these species in infected patients, and between sites in infected patients and comparable sites in non-infected patients. In addition, some baseline clinical and microbiological data for these patients are presented. The distribution of PI and GI scores tended to be highest for molars, with the remaining teeth having similar scores. Probing depth measurements were greater for mesio-distal than oro-vestibular recordings. A bilaterally symmetrical pattern of increasing probing depth was noted from the midline distally on mesio-distal as well as oro-vestibular surfaces. Sites positive for the target organisms listed above tended to have greater probing depths (mean: 4.14 ± 0.1 mm) than non-infected sites in the same patients (mean: 3.76 ± 0.1 mm) or comparable sites in non-infected patients (mean: 3.54 ± 0.1 mm).

Relatively little information has been published on the periodontal status of patients previously treated for periodontitis and subsequently maintained in satisfactory periodontal health by routine prophylactic visits. Some reports have suggested that patients maintained on a regular program of periodontal prophylaxes remain free of disease recurrences,1-3 while other reports indicate that some disease recurrence can be expected.4-7 To a large extent, the apparently contradictory results are due to the manner in which the resulting data are analyzed. Other reports have indicated that the incidence of disease recurrence varies among subjects, with a relatively small group of subjects accounting for the bulk of the recurrences.8-10

Poor oral hygiene is considered by some as an important contributing factor in recurrent disease,2,11-15 whereas others find little correlation between oral hygiene status and/or gingivitis and the recurrence of periodontitis.15-17 Most reports indicate that multi-rooted teeth are generally more difficult to maintain than single rooted teeth.3,7,10,13,18 Although there is little doubt that regular maintenance visits are essential for successful treatment outcome,19-20 there is some controversy as to whether the original treatment influences the success of the maintenance program. Some reports have suggested that surgical compared to nonsurgical therapy may be more effective in facilitating subsequent maintenance,21 while others have indicated that the type of therapy has little impact on long-term maintenance,20 or that surgical therapy may actually result in greater loss of attachment in the long run.4

In a one-year longitudinal study of patients previously treated for adult periodontitis, Listgarten and Levin22 reported that base line proportions of certain bacterial morphotypes in the subgingival area were predictive of future recurrences of disease in a treated population from which regular periodontal prophylaxes were withheld. In a later report, Listgarten et al.23 indicated that the reliability of microscopic monitoring of subgingival bacterial morphotypes as a predictor of future disease recurrence fell sharply in subjects undergoing regular trimonthly periodontal prophylaxes. Since studies by Slots24 and Slots et al.25 indicated that certain cultivable bacterial species might be associated with progressive periodontitis, a longitudinal prospective study has been started to determine whether Actinobacillus actinomyctemcomitans, Bacteroides gingivalis and Bacteroides intermedia could serve as predictors of the future clinical course of the disease in a treated population. In this report we present the

* Department of Periodontics and General Clinical Research, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA.
clinical and microbiological characteristics at base line of the population to be studied.

MATERIALS AND METHODS

Ninety-eight adults, at least 25 years of age, were recruited for this study. All subjects had been treated for moderate to advanced periodontitis of the adult type, and subsequently enrolled in a maintenance program consisting of visits spaced from one to several months apart. Approximately 75% of the patients in this study had been enrolled in a maintenance program for periods of 5 years or more. All subjects had been in maintenance care for at least one year.

Medical criteria for exclusion included histories of rheumatic fever, diabetes mellitus, venereal disease, blood dyscrasias, anomalies of the immune system, or unusual susceptibility to infections that would require the routine use of prophylactic antibiotics. Subjects regularly taking medication potentially capable of affecting the qualitative composition of the periodontal microbiota were also excluded. Patients with dental records suggestive of localized juvenile periodontitis were excluded from the study.

All subjects had at least 18 teeth, with at least 4 teeth in each quadrant. All patients exhibited evidence of previous periodontal breakdown, as demonstrated by radiographic evidence of alveolar bone loss, and records of probing depth measurements prior to treatment of 5 mm or more affecting at least one tooth in at least 3 of 4 quadrants. The disease in these patients had been of sufficient severity to warrant surgical pocket reduction in at least two quadrants.

After they gave informed consent, volunteers for the study underwent a comprehensive examination which consisted of the following.

Oral Hygiene Status

The presence of supragingival plaque was assessed on all mesial, distal, vestibular (buccal, labial) and oral (lingual, palatal) surfaces, according to the criteria of the Plaque Index (PII) scoring system which is based on scores in the range of 0 to 3.

Inflammation

Gingival inflammation was assessed on the same surfaces described above using the Gingival Index (GI) scoring system. Like the PII scores, GI scores range from 0 to 3.

Probing Depth Measurements

Probing depth (PD) was recorded to the nearest mm using a probe calibrated in mm with special markings at 5, 10, 15, and 20 mm. The probe was introduced, with a force approximating 50 pounds, until resistance was felt. The length of the probe tip to the gingival margin was recorded. The two examiners practiced probing on a top loading balance and with a Yeaple calibrated probe until the desired force could be routinely applied to in vivo measurements. However, routine measurements were not carried out with the Yeaple probe. Measurements were taken from the same surfaces described above. The probing depth chosen was always the deepest site recorded on the tooth surface measured.

Attachment Level Measurements

Attachment levels (AL) were determined by probing the same sites described above and measuring the distance from the probe tip to a fixed reference consisting of a trimmed thermoplastic occlusal stent.

The two examiners were calibrated prior to the beginning of the experiment in training sessions in which typical clinical slides of periodontal patients on maintenance were reviewed, and by performing actual examinations on the same subjects. Additional training sessions are held every 3 months.

Microbiological Techniques

The microbiological study was focused on A. actinomycetemcomitans, B. gingivalis, and B. intermedius, which served as target species. However, this report also describes the occurrence of other species, namely Bacteroides melaninogenicus, Eikenella corroden and Capnocytophaga. The distribution of E. corroden was studied in a randomly selected subset of patients.

Microbiological sampling, processing of specimens, selection of microbiological media, and incubation conditions have been described in a previous paper. Briefly, subgingival samples were obtained by paper points from individual periodontal sites or were pooled from 6 deep periodontal pockets. The paper points were kept in place for 10 seconds before transfer to the Möller's VMGA III transport medium, in a vial containing 3 mm glass beads. Dispersion of microorganisms was carried out with a Vortex mixer at the maximal setting for 60s, and 0.1 ml aliquots of 10-fold serial dilutions plated within 2 hours of collection.

The primary isolation media included a non-selective enriched brucella blood agar (EBBA) consisting of 4.3% brucella agar, supplemented with 0.3% Bacto-agar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, 0.00005% menadione, 0.2% sodium formate, and 0.3% sodium fumarate. A selective medium was inoculated for the selective recovery of black-pigmented Bacteroides, TSBV medium for the selective recovery of A. actinomycetemcomitans, Walker's selective medium for E. corroden, and a selective medium (TBBP) for

* Hu-Friedy manufacturing

† Vine Valley Research, Middlesex, NY.

‡ BBL Microbiology Systems, Cockeysville, MD.
Capnocytophaga spp. EBBA plates and the selective plates for black-pigmented Bacteroides were incubated at 35°C in a Coy anaerobic chamber containing 85% N₂, 10% H₂, and 5% CO₂ for 10 days. The TSBV and TBBP media were incubated at 35°C in 10% CO₂ and 90% N₂ for 4 days.

The black-pigmented Bacteroides spp. and Capnocytophaga spp. were monitored on nonselective as well as selective media. The proportional recovery of various species was determined by comparing the recovery of the test bacteria to the total cultivable microbiota on nonselective medium. Bacterial isolates were tentatively identified by the criteria and techniques of Slots. Microbiological Screening and Experimental Design

Microbiological samples were collected at least 3 weeks after the initial clinical examination from sites with the greatest probing depth on 2 teeth in each jaw sextant. Each site provided a single sample, so that 12 individual samples were available for each subject. In addition, the pocket in each sextant with the greatest probing depth provided a set of 6 samples that were pooled to obtain a single pooled sample for each subject. After the samples were processed an attempt was made to identify subjects with at least one pocket positive for A. actinomycetemcomitans, B. gingivalis or B. intermedius, at levels equal to or exceeding 0.01%, 0.1%, and 2.5% of the total cultivable flora, respectively. In cross-sectional studies by Bragg et al. these bacterial recovery levels were suggested to place infected sites at increased risk for further periodontal breakdown.

Subjects with at least two such sites were designated as “Positive” subjects while the remaining subjects were considered to be “Negative” or “Control” subjects, as shown below:

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Sampled sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive subjects</td>
<td>Positive (Target) sites</td>
</tr>
<tr>
<td>Negative subjects</td>
<td>Negative (Internal control) sites</td>
</tr>
<tr>
<td></td>
<td>Negative (External control) sites</td>
</tr>
</tbody>
</table>

Microbiological screening of the subjects was continued until 98 subjects were recruited. After Positive subjects were identified, 6 sites previously sampled microbiologically were identified so that up to 3 were positive and up to 3 were negative for the test bacteria. The positive and negative sites in each of 72 Positive subjects were designated as “Target” and “Internal Control” sites respectively. In the 26 Negative subjects, 6 negative sites were selected, as previously described, to serve as “External Control” sites.

This experimental design allows comparisons to be made between Positive and Negative subjects, between Target and Internal Control sites within Positive subjects, and between Target and Internal Control sites in Positive subjects or External Control sites in Negative subjects.

All subjects were placed on a trimonthly recall schedule for prophylaxes. They will be reexamined every 6 months, and samples collected for additional microbiological assays, and for immunological assays to be reported separately. Disease recurrence at any site will be identified as a probing depth increase of 3 mm or more from base line, or as an increase in probing depth of 2 mm or more, together with a loss of attachment of 2 mm or more from the reference stent.

The data were collected on the CLINFO Clinical Data Management System in the General Clinical Research Center at the School of Dental Medicine. Descriptive statistics and comparative analyses were obtained using CLINFO and BMDP 87 software. Generally, positive versus Negative subjects contrasts on clinical indices were made with the two-sample t-test, while contrasts on microbiological responses were made more appropriately with the Mann–Whitney test, because of their heavily right skewed distributions. The within-subject Target versus Control studies were made with the paired t-test. All significant P-values reported are two-tailed, and are supported by both parametric and nonparametric procedures, if available. Homogeneity for Positive and Negative subjects with respect to the number of teeth positive for various microbial species was examined with the chi-square test statistic.

RESULTS

The clinical data which follow are based on the baseline examination of 98 subjects. One Positive subject without any suitable control sites was excluded from all internally controlled analyses. To simplify the presentation of the clinical data, mesial and distal measurements have been averaged, since these measurements were similar to each other. Likewise, oral and vestibular measurements have been averaged and will be presented as the mean oro-vestibular measurements for each criterion and each tooth.

Only base line PII, GI, and PD measurements will be presented, since AL measurements from an arbitrarily determined fixed reference on an occlusal stent at a single point in time are not particularly informative. However, by subtracting base line measurements for AL from subsequent recordings, it will be possible to estimate attachment gain or attachment loss at the various sites monitored longitudinally.

PII scores followed a similar distribution for both oro-vestibular and mesio-distal surfaces (Figs. 1 and 2). The scores tended to be highest for molars with the remaining teeth showing similar scores. Curiously, the GI scores did not follow this pattern. The GI scores were more or less uniform throughout the dentition (Figs. 3 and 4). If anything, the mesio-distal molar scores tended to be somewhat lower than the mesio-distal scores of the remaining dentition (Fig. 3).

The PD measurements tended to be greater for me-
siodistal (Fig. 5) than oro-vestibular surfaces (Fig. 6). In addition, there appeared to be a gradual increase in PD measurements from the midline posteriorly, whether oro-vestibular or mesio-distal surfaces were considered. The distribution of PD measurements followed a bilaterally symmetrical pattern.

Following the selection of Positive and Negative subjects and the selection of Target and Control sites in Positive subjects, the mean PII, GI, and PD measurements were recalculated for these subgroups, as shown in Table 1. It is of interest to note that the mean PD of Target sites (4.14 ± 0.1 mm) was significantly greater than that of the Internal Control sites in the Positive subjects (3.76 ± 0.1 mm) or the External Control sites in the Negative subjects (3.54 ± 0.1 mm).

Because the subgrouping described above was carried out on the basis of bacterial screenings of each site for the presence of critical levels of specific species, it is not surprising to find greater levels of *A. actinomycetem-comitans, B. gingivalis, and B. intermedius* in the Target sites of Positive subjects than in the Control sites of either Positive or Negative subjects (Table 2). No significant differences were found among the site subgroups for *Eikenella corrodens, Capnocytophaga* or *Bacteroides melaninogenicus*.

In further analyses, subgroups of subjects who were positive for any of the following organisms, *A. actinomycetemcomitans, B. intermedius, B. gingivalis, B. melaninogenicus, Capnocytophaga* species, and *E. corrodens* were compared with respect to PD at Target and Internal Control sites. Thus, this analysis did not include the negative subjects. Mean probing depth at Target sites was significantly greater than the mean PD at Control sites for *A. actinomycetemcomitans* (P < 0.02), *B. intermedius* (P < 0.001), *B. melaninogenicus* (P < 0.01) and *Capnocytophaga* (P < 0.01) (Table 1). Since *B. gingivalis* was only observed in 11 subjects, no comparisons of PD measurements were carried out
between Target sites positive for this organism and Control sites.

Table 3 reports the distributions of the microbial species listed in Table 2 by number of infected sites. In some instances, the number of infected sites exceeds the 3 sites designated as Target sites. This is explained by the fact that the proportions of indicator bacteria in some sites were below the critical levels needed for sites to be considered positive for these species and, therefore, suitable for Target site designation.

**DISCUSSION**

Both P1I and GI scores were relatively low, indicating that these subjects maintained a fairly high standard of oral hygiene and, as a result, developed little gingivitis. The difficulty experienced by many patients in cleaning the second molars may explain the tendency for the molars to exhibit higher plaque scores.

The distribution of PD measurements in treated pa-

Table 1

<table>
<thead>
<tr>
<th>Mean Clinical Measurements at Base Line</th>
<th>Experimental groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target (n = 72)</td>
<td>Internal control (n = 71)</td>
</tr>
<tr>
<td>x ± SE</td>
<td>x ± SE</td>
</tr>
<tr>
<td>Plaque Index</td>
<td>0.57 ± 0.05</td>
</tr>
<tr>
<td>Gingival Index†</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>Probing Depth‡</td>
<td>4.14 ± 0.1</td>
</tr>
</tbody>
</table>

* See Methods and Materials
† GI scores were significantly greater in Target vs. Internal control sites in subjects positive for *Capnocytophaga (P < 0.05).
‡ PD measurements were significantly greater in Target vs. Control sites in subjects positive for either *A. actinomycetemcomitans (P < 0.02), B. intermedia (P < 0.001), Capnocytophaga (P < 0.01) or B. melaninogenicus (P < 0.01).
§ P < 0.01.
|| P < 0.001.

patients on maintenance differs from that reported for untreated periodontitis by Löe and co-workers. These authors reported that the teeth with the greatest PD in untreated Sri Lankan and Norwegian populations were the maxillary first molars and lower incisors, while the mandibular canines and first premolars were the teeth with the least PD. The data reported by Spolsky for US residents shows the prevalence of periodontal disease to be similarly distributed. This is in contrast to our findings which suggests a pattern of progressively greater PD as the distance of the teeth from the midline increases. Presently, there is no obvious explanation for the observed differences in the distribution of PD between untreated and treated patients.

Our findings do not necessarily mean that tooth loss from periodontal disease in patients or maintenance will follow a similar pattern. Indeed, reports from Hirschfeld and Wasserman, Goldman et al. and our own research group suggest that rate of tooth loss is not strictly associated with distance of a tooth from the midline, although molars are generally more likely to be lost than the remaining teeth.

The bilaterally symmetrical distribution of PD, coupled with the symmetrical anatomy of the jaws and teeth, are compatible with the reports of a bilaterally symmetrical distribution of periodontal attachment loss in other populations. These findings argue against the concept of periodontitis recurrence as being a random phenomenon with all sites (surfaces) at equal risk of developing periodontitis.

The observation that Target sites in Positive patients had PD measurements significantly greater than those of Internal or External Control sites supports the previously reported correlation between *A. actinomycetemcomitans, B. intermedia*, and *B. gingivalis* and increased loss of attachment. The association of *Capnocytophaga* with greater GI scores and increased PD in Target sites of Positive subjects may reflect a similar
Table 2
Mean Proportions of Selected Bacterial Species at Baseline Including the 3 Target Species *A.
actinomycetemcomitans* (Aa), *B. gingivalis* (Bg), and *B. intermedius* (Bi)

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Experimental groups†</th>
<th>Target x ± SE (n)</th>
<th>Internal control x ± SE (n)</th>
<th>External control x ± SE (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aa</em></td>
<td></td>
<td>0.012 ± 0.003 (72)</td>
<td>0.000 ± 0.000 (71)</td>
<td>0.000 ± 0.000 (26)</td>
</tr>
<tr>
<td><em>Bg</em></td>
<td></td>
<td>0.003 ± 0.001 (72)</td>
<td>0.000 ± 0.000 (71)</td>
<td>0.000 ± 0.000 (26)</td>
</tr>
<tr>
<td><em>Bi</em></td>
<td></td>
<td>0.075 ± 0.009 (72)</td>
<td>0.000 ± 0.000 (71)</td>
<td>0.000 ± 0.000 (26)</td>
</tr>
<tr>
<td><em>Ec</em></td>
<td></td>
<td>0.000 ± 0.000 (58)‡</td>
<td>0.000 ± 0.000 (56)‡</td>
<td>0.000 ± 0.000 (21)‡</td>
</tr>
<tr>
<td><em>C</em></td>
<td></td>
<td>0.012 ± 0.003 (72)</td>
<td>0.014 ± 0.003 (71)</td>
<td>0.004 ± 0.001 (26)</td>
</tr>
<tr>
<td><em>Bm</em></td>
<td></td>
<td>0.007 ± 0.003 (72)</td>
<td>0.003 ± 0.003 (71)</td>
<td>0.001 ± 0.000 (26)</td>
</tr>
</tbody>
</table>

*Ec: E. corrodens; C: Capnocytophaga; *Bm*: B. melaninogenicus.
† See Methods and Materials.
‡ *Ec* was studied in a randomly selected subset of patients. This accounts for the lower n value for this microorganism.
§ *P < 0.001.
II *P < 0.05.

Table 3
Distribution of Selected Microbial Species in the Experimental Population by Number of Sites per Subject Positive for the Species Tested

| No. of subjects with 0-6 teeth positive for *A. actinomycetemcomitans* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 71                   |
| Control subjects             | 26 0 0 0 0 0            | *P < 0.001*          |

| No. of subjects with 0-6 teeth positive for *B. gingivalis* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 71                   |
| Control subjects             | 26 0 0 0 0 0            | *P < 0.05*           |

| No. of subjects with 0-6 teeth positive for *B. intermedius* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 71                   |
| Control subjects             | 22 2 2 0 0 0            | *P < 0.001*          |

| No. of subjects with 0-6 teeth positive for *E. corrodens* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 53                   |
| Control subjects             | 18 1 0 1 0 1            | NS                  |

| No. of subjects with 0-6 teeth positive for *Capnocytophaga sp.* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 71                   |
| Control subjects             | 7 7 5 3 2 2 0           | NS                  |

| No. of subjects with 0-6 teeth positive for *B. melaninogenicus* | No. of positive teeth | Total Significance |
|---|-------------------------------|-----------------------|-------------------|
| Positive subjects            | 0 1 2 3 4 5 6           | 71                   |
| Control subjects             | 17 7 2 0 0 0            | NS                  |

457
relationship between this species and inflamed sites with increased PD. It may also be due to the predilection of Capnocytophaga to be associated with A. actinomyces-
temcomitans in infected sites (Pearson r = 0.27, P < 0.02, own unpublished data). No relationship could be demonstrated between gingivitis and E. corrodens or B. melaninogenicus; however, Target sites had a significantly increased PD when compared with Control sites in subjects positive for either A. actinomyces-
temcomitans, B. intermedius, B. melaninogenicus or Capnocy-
tophaga species.

Even in sites likely to harbor some of the species monitored, the majority of sites were often negative for these species. As a result, the mean proportions of these species reported for the various experimental subgroups appear much lower than they might be if only sites positive for these species had been examined.

ACKNOWLEDGMENTS
This research was supported by the National Institute of Dental Research grant RO1-DE06085, a grant from the Colgate-Palmetto Company, and General Clinical Research Center Grant RR-01224 to the School of Dental Medicine, University of Pennsylvania.

REFERENCES
27. Löe H, Silness J. Periodontal disease in pregnancy. I. Preva-
28. Sweeney EA; Alcoforado GAP, Nyman S, Slots J. Prevalence and microbiology of localized prepubertal periodontitis. Oral Micro-
30. Zambon JJ, Reynolds HS, Slots J. Black-pigmented Bacterio-
31. Slots J. Selective medium for isolation of Actinobacillus acti-
34. Slots J. Rapid identification of important periodontal micro-
36. Bragd L, Dahlén G, Wikström M, Slots J. The capacity of Actinobacillus actinomycescomitans, Bacteroides gingivalis and Bacteroides intermedius to indicate progressive periodontitis; a re-


Send reprint requests to: Dr. Max Listgarten, 4001 Spruce St., Philadelphia, PA.

Accepted for publication February 28, 1989.