

Clinical and microbiological changes associated with the use of combined antimicrobial therapies to treat “refractory” periodontitis

A. D. Haffajee, N. G. Uzel,
E. I. Arguello, G. Torresyap,
D. M. Guerrero and S. S. Socransky
Department of Periodontology, The Forsyth
Institute, Boston, MA, USA

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Abstract

Background: The present investigation examined clinical and microbial changes after a combined aggressive antimicrobial therapy in subjects identified as “refractory” to conventional periodontal therapy.

Method: Fourteen subjects were identified as “refractory” based on full-mouth mean attachment loss and/or >3 sites with attachment loss ≥ 3 mm following scaling and root planing (SRP), periodontal surgery and systemic antibiotics. After baseline monitoring, subjects received SRP, locally delivered tetracycline at pockets ≥ 4 mm, systemically administered amoxicillin (500 mg, t.i.d. for 14 days)+metronidazole (250 mg, t.i.d. for 14 days) and professional removal of supragingival plaque weekly for 3 months. Subjects were monitored clinically every 3 months post-therapy for 2 years. Subgingival plaque samples were taken at the same time points from the mesial aspect of each tooth and the levels of 40 subgingival taxa were determined using checkerboard DNA–DNA hybridization. Mean levels of each species were averaged within a subject at each visit. Significance of changes in clinical and microbiological parameters over time were evaluated using the Friedman or Wilcoxon signed ranks test.

Results: On average, subjects showed significant improvements in all clinical parameters after therapy. Mean (\pm SEM) full-mouth pocket depth reduction was 0.83 ± 0.13 mm and mean attachment level “gain” was 0.44 ± 0.12 at 24 months. Clinical improvement was accompanied by major reductions in multiple subgingival species during the first 3 months of active therapy that were maintained for most species to the last monitoring visit. Reductions occurred for three *Actinomyces* species, “orange complex” species including *Campylobacter showae*, *Eubacterium nodatum*, three *Fusobacterium nucleatum* subspecies, *Peptostreptococcus micros*, *Prevotella intermedia* as well as the “*Streptococcus milleri*” group, *Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*. Subjects differed in their response to therapy; six modest response subjects exhibited less attachment level gain and were characterized by reductions in the microbiota from baseline to 3 months, but re-growth of many species thereafter.

Conclusions: The combined antibacterial therapy was successful in controlling disease progression in 14 “refractory” periodontitis subjects for 2 years.

Key words: attachment level; periodontal diseases; pocket depth; “refractory” periodontitis; subgingival microbiota; treatment

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In an attempt to define some of the microbial or host factors that might lead to a poor periodontal treatment response, Colombo et al. (1998a) treated 94 adult subjects with periodontitis using a series of treatments that included scaling and root planing (SRP), periodontal surgery and systemically administered antibiotics. Twenty-eight subjects were classified as poor responders or "refractory" to treatment based on mean full-mouth attachment level loss and/or >3 sites exhibiting >2.5 mm loss of attachment within 1 year post-therapy. Sixty-six successfully treated subjects exhibited mean full-mouth attachment-level gain and <3 sites with attachment loss >2.5 mm. After completion of treatment, the subjects characterized as refractory, were placed on a maintenance program consisting of 3-month recall for SRP and reinforcement in home-care procedures. In spite of this program and well-performed home care, the subjects continued to exhibit disease progression as measured by new attachment loss >2.5 mm at multiple sites during this maintenance phase. Since conventional therapy, followed by regular periodontal maintenance visits was ineffective in stopping disease progression, it was felt that more aggressive means would have to be employed to control disease in these individuals.

The reason that a subject is "refractory" is poorly understood. A number of differences have been described in the pre-therapy subgingival microbiota of refractory subjects when compared with successfully treated subjects (Haffajee et al. 1988, Magnusson et al. 1991, Colombo et al. 1998a, Socransky et al. 2002), and there may be differences in the host response (Magnusson et al. 1991, MacFarlane et al. 1992, Reinhardt et al. 1993, Hernichel-Gorbach et al. 1994, Jin et al. 1995, Kurdowska et al. 2003). The subjects in the Colombo et al. (1998a) study exhibited levels of presumed periodontal pathogens such as *Tannerella forsythensis* (*Bacteroides forsythus*), *Porphyromonas gingivalis* and *Treponema denticola* after conventional periodontal treatment which were comparable with the reductions of these species observed in successfully treated subjects. It was hypothesized that the "refractory" subjects either had a poor host response, unusually virulent subgingival pathogens or both. In any of these situations, it seemed essential that the level of the colonizing subgingival

species be driven to even lower levels. Thus, the purpose of the present investigation was to determine whether an aggressive antimicrobial therapy that combined multiple previously successful antimicrobial approaches could: (a) lower the levels of subgingival colonizing species from their already lowered levels and (b) whether this microbial effect would lead to stability of the supporting structures of the tooth.

Material and Methods

Subject population

Fourteen subjects with "refractory" periodontitis who had complete clinical and microbiological data 24 months post-therapy were included in this report. Eight of these subjects were from the original Colombo et al. (1998a) study while six subjects were included based on the same failure to respond to a series of therapies described in the original Colombo et al. (1998a) paper. The study was approved by The Forsyth Institute Investigational Review Board and all subjects were informed of the nature, potential risks and benefits of study participation and signed informed consent prior to entry into the study. The subjects ranged in age from 23 to 68 years. Exclusion criteria included pregnancy and any systemic condition that might have affected the progression of periodontitis. No subject with localized juvenile periodontitis or acute necrotizing ulcerative gingivitis was included in the study. Smoking history was obtained using a questionnaire. All answers were reviewed with the subject by a member of the study team. The baseline clinical parameters for the subject group are presented in Table 1.

Clinical measurements

Measures of plaque accumulation (0/1), overt gingivitis (0/1), bleeding on probing (BOP, 0/1), suppuration (0/1), probing pocket depth and probing attachment level were taken at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars at a baseline visit. Pocket depth and attachment-level measurements were made using a North Carolina probe (Hu-Friedy, Chicago, IL, USA). The pocket depth and attachment-level measurements were repeated and the means of the pairs of measurements were used in the analyses (Haffajee et al. 1983). Clinical measurements were taken pre-therapy and at 3-month intervals for 2 years post-therapy (a total of nine visits).

Microbiological assessment

Subgingival plaque samples were taken from the mesio-buccal aspect of all teeth except third molars in each subject pre-therapy and at 3-month intervals for 2 years post-therapy. Counts of 40 subgingival species were determined in each plaque sample using a modification (Haffajee et al. 1997) of the checkerboard DNA-DNA hybridization technique (Socransky et al. 1994). In brief, after the removal of supragingival plaque, subgingival plaque samples were taken using individual sterile Gracey curettes (Hu-Friedy) from the mesial aspect of each tooth. The samples were placed in separate Eppendorf tubes (Fisher Scientific, Boston, MA, USA) containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and 0.15 ml of 0.5 M NaOH was added. The samples were lysed and the DNA placed in lanes on a nylon membrane using a

Table 1. Mean (\pm SD) baseline clinical parameters of the 14 "refractory" subjects

| | Mean \pm SD | Range |
|----------------------------|-----------------|-----------|
| number of subjects | 14 | |
| age (years) | 46 \pm 11 | 23–68 |
| number of missing teeth | 4.5 \pm 4.4 | 0–13 |
| % males | 50 | |
| mean pocket depth (mm) | 3.61 \pm 0.38 | 2.98–4.13 |
| mean attachment level (mm) | 4.42 \pm 1.12 | 3.19–6.82 |
| % of sites with | | |
| plaque | 66 \pm 20 | 26–91 |
| gingival redness | 59 \pm 18 | 27–88 |
| bleeding on probing | 33 \pm 15 | 8–59 |
| suppuration | 4.9 \pm 5.7 | 0–17.7 |
| % current smokers | 57 | |
| % past smokers | 22 | |
| pack years | 27.2 \pm 32.7 | 0–86.1 |

Minislot device (Immunetics, Cambridge, MA, USA). After fixation of the DNA to the membrane, the membrane was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 40 subgingival species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and chemi-fluorescence detection. Signals were detected using AttoPhos substrate (Amersham Life Science, Arlington Heights, IL, USA) and were read using a Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA, USA), a computer-linked instrument that reads the intensity of the fluorescence signals resulting from the probe-target hybridization. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals were evaluated using the Storm Fluorimager and converted to absolute counts by comparison with the standards on the same membrane. Failure to detect a signal was recorded as zero. A total of 2953 subgingival plaque samples (mean 23.4 samples per subject per visit) were evaluated.

Treatment

After baseline clinical and microbiological monitoring, subjects received full-mouth SRP under local anesthetic. Quadrants were scaled at approximately weekly intervals so that this phase of treatment was completed within 1 month. Tetracycline-loaded fibers (Actisite; Procter and Gamble, Cincinnati, OH, USA) were placed at pockets >4 mm after scaling in a given quadrant. The fibers were removed 1 week later at the next scaling visit or 1 week after completion of SRP. The subjects also received systemically administered amoxicillin at the dosage of 500 mg t.i.d. and metronidazole at the dosage of 250 mg t.i.d. for 14 days starting at the first scaling visit. Finally, subjects received professional supragingival plaque removal once a week for 3 months after the completion of the SRP phase (Ximenez-Fyvie et al. 2000). After the active phase of therapy, subjects received maintenance SRP and reinforce-

ment of home-care procedures every 3 months.

Data analysis

Clinical data including measures of plaque accumulation, gingival redness, BOP, suppuration, as well as duplicate pocket depth and attachment-level measurements were available from up to 168 sites in each subject from the pre-therapy visit and eight post-therapy visits. Microbiological data available for each subject at the same time points were the counts of each of the 40 test species from up to 28 subgingival plaque samples per subject. Clinical measurements and the counts of each species were averaged within a subject at each visit and the significance of differences over time for each parameter was determined using the Friedman test. Significance of differences between pairs of visits was determined using the Wilcoxon signed ranks test.

Results

Clinical findings

A total of three teeth were extracted from 14 subjects during the 24 months of the study; two in one subject and one tooth in a second subject. This translated to a rate of 0.1 teeth per subject per year. Full-mouth mean clinical parameters were significantly improved

post-therapy (Fig. 1). Major reductions in plaque accumulation, gingival redness, BOP and suppuration occurred by the 3-month monitoring visit and were sustained to 24 months. The majority of the reduction in mean pocket depth and attachment level had occurred by 6 months post-therapy and the mean improvements continued to the 24-month monitoring visit. Significant reductions in mean pocket depth and BOP could be observed at sites subset into initial pocket depth categories of <4, 4–6 or >6 mm. Significant reductions of mean periodontal attachment measurements, suppuration and gingival redness occurred only at sites with baseline pocket depths of 4–6 or >6 mm (data not shown).

Improvement in clinical attachment level occurred for most subjects by 6–9 months and continued out to 24 months (Fig. 2). Major reduction in pocket depth occurred by 6–12 months, but pocket depth reduction continued for some subjects beyond this time frame (data not shown). The subjects were subset into two groups, good responders ($n = 8$) and modest responders ($n = 6$) based on their mean full-mouth attachment-level change from baseline to 24 months (Fig. 2). The change in mean (\pm SEM) full-mouth attachment level for the modest responders from baseline to 24 months was 0.0 ± 0.04 mm (range: "gain" of 0.13 to a loss of 0.08 mm). The corresponding values for the good responders was a mean "gain" of 0.77 ± 0.11 mm (range: 0.39–1.27 mm).

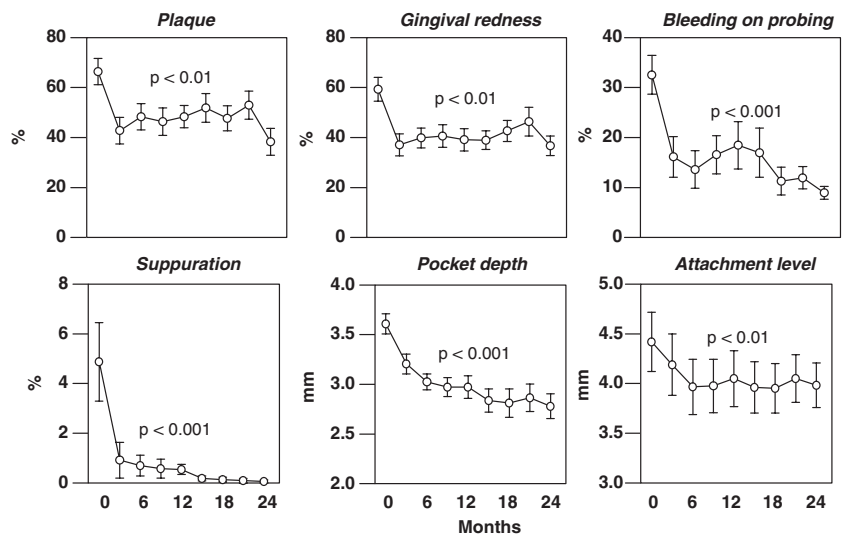


Fig. 1. Plots of mean clinical parameters (\pm SEM) pre-therapy and at 3-month intervals post-therapy for the 14 "refractory" subjects. Significance of differences over time for the mean clinical parameters was evaluated using the Friedman test. Note that the values on the y-axis for pocket depth and attachment level do not start at 0.

Microbiological findings

To characterize the nature of the subgingival microbiota in the “refractory” periodontitis subjects in this study at baseline, the mean pre-therapy counts ($\times 10^5$) were plotted for these subjects and compared with baseline counts from 360 untreated periodontitis subjects from the same geographic area (Fig. 3). There was a marked difference in mean microbial counts for 13 of the taxa evaluated. In particular, members of the green, orange and red complexes as well as some of the species in the “other” category were significantly elevated in the periodontitis subjects compared with the refractory subjects. Notable exceptions were members of the genus *Actinomyces*, *Selenomonas noxia* and all three members of the “*Streptococcus milleri* group”, *Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*.

The mean counts prior to and at different time points post-therapy for the 40 subgingival species in the 14

“refractory” subjects are presented in Fig. 4. At 3 months post-therapy, there was a marked decrease in the counts of

the majority of the test species. The counts of many, but not all, of the species increased over time. Increases

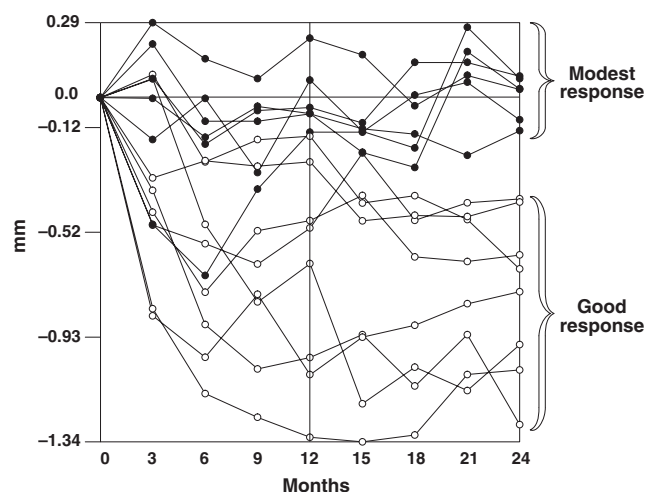


Fig. 2. Mean attachment-level change from baseline to each time point to 24 months for individual subjects. The values above the horizontal zero line represent worsening, while values below this line represent improvement in this parameter. The subjects highlighted by solid circles were considered to exhibit a modest therapeutic response while the subjects delineated by open circles exhibited a good therapeutic response.

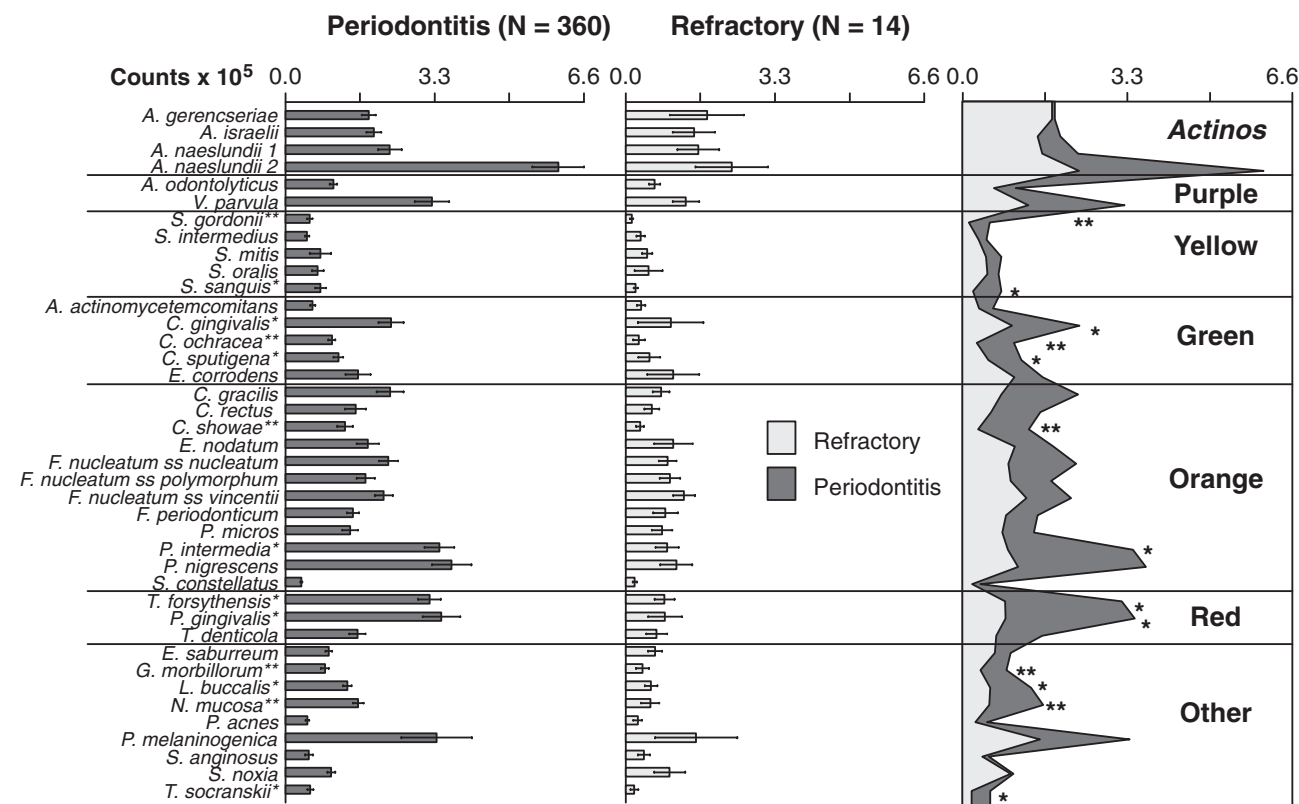


Fig. 3. Bar charts of mean counts ($\times 10^5$, +SEM) of individual species in baseline subgingival plaque samples taken from 360 chronic periodontitis and 14 “refractory subjects”. The bars represent the mean counts and the whiskers indicate the standard error of the mean. Mean counts for each species were computed in each subject and then averaged across subjects in the two clinical groups. Significance of differences between groups was sought using the Mann–Whitney test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The species were ordered and grouped according to the complexes described by Socransky et al. (1998). The right panel presents the same mean data as profiles. The dark gray profile represents the mean data for the periodontitis subjects and the light gray profile represents the mean data for the “refractory” subjects.

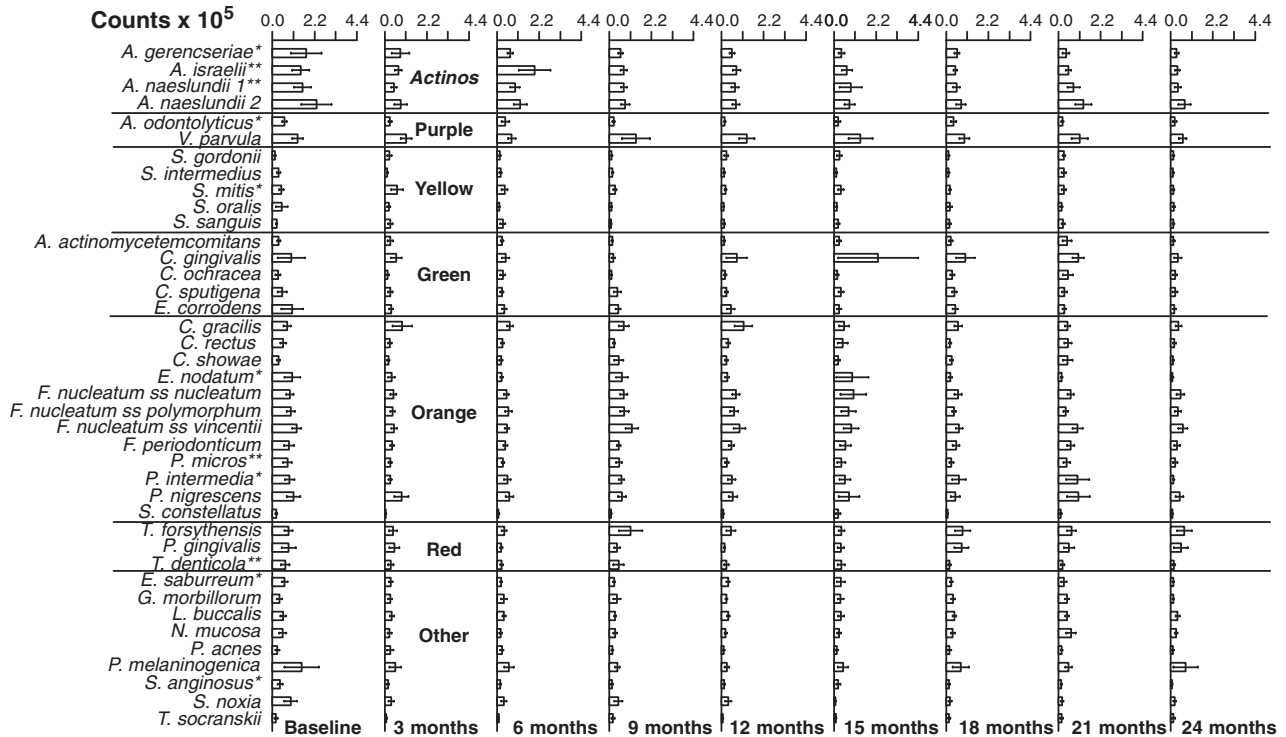


Fig. 4. Bar charts of the mean counts ($\times 10^5$, +SEM) of the 40 test species in subgingival plaque samples taken pre-therapy and at 3-month intervals to 24 months post-therapy. The combined therapy was performed between baseline and 3 months. Mean counts for each species were computed for a subject for each visit and then values were averaged across the 14 subjects at each time point. The whiskers indicate the SEM. Significance of differences over time was sought using the Friedman test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The species were ordered and grouped according to the complexes described by Socransky et al. (1998).

were noted for many members of the orange and red complexes in these overall data. The *Actinomyces* remained at lowered levels throughout the 2 years of monitoring.

The most noticeable shifts in the microbiota took place from baseline to 3 months, followed by a prolonged stable phase (Fig. 5). Major reductions were observed between baseline and 3 months for the *Actinomyces* species, many of the orange complex species as well as *T. forsythensis*, *S. anginosus*, *S. noxia* and *Treponema socranskii*. The period from 3 to 24 months was characterized by a modest increase in some of the taxa, although these increases were not statistically significant (Fig. 5). Notably, the *Actinomyces* and the members of the "S. milleri group" remained at low levels during this time period.

Subjects were subset into those exhibiting a good or moderate clinical response based on mean attachment level change at 24 months (Fig. 2). Figs 6 and 7 present the microbial profiles from baseline to 3 months (Fig. 6) and from 3 to 24 months (Fig. 7) in the two subjects groups. From baseline to 3 months, there was a marked reduction in

counts of the *Actinomyces* species, and some members of the orange complex in both groups (Fig. 6). Additionally, counts of *T. forsythensis*, *T. denticola*, *T. socranskii*, and the "milleri" streptococci, *S. anginosus*, *S. constellatus* and *S. intermedius* were significantly reduced in the good response group. From 3 to 24 months, the mean counts of most of the test species in the good response group remained at the reduced levels or continued to decline (*Actinomyces israelii*, *Veillonella parvula* and *S. anginosus*). In contrast, counts of many the test species increased in the modest response group, including members of the red and orange complexes as well as *Prevotella melaninogenica*, *S. noxia* and *T. socranskii*, although these increases were not statistically significant.

Discussion

The results of the present investigation indicated that most subjects considered to be "refractory" after a series of conventional periodontal therapies could be successfully treated using a combination

of mechanical and antimicrobial therapies. The chosen therapies were put together with the idea that combining therapies that had different modes of action would lower the subgingival microbiota to levels below the already lowered levels that resulted from the subjects' previous periodontal therapy. This goal appeared to have been achieved. Further, the reduction in levels of subgingival species was accompanied by an improvement in clinical status that could be documented for 2 years.

The notion of "refractory" periodontal disease has been around for a number of years and the major emphasis of most studies to date has been on determining the reasons for the poor response to conventional therapies. Proposed reasons for "refractory" disease have included differences in the subgingival microbiota (Haffajee et al. 1988, Magnusson et al. 1991, Colombo et al. 1998a, Socransky et al. 2002), differences in host response (Magnusson et al. 1991, MacFarlane et al. 1992, Reinhardt et al. 1993, Hernichel-Gorbach et al. 1994, Jin et al. 1995, Colombo et al. 1998b, Kurdowska et al. 2003) and differences in environ-

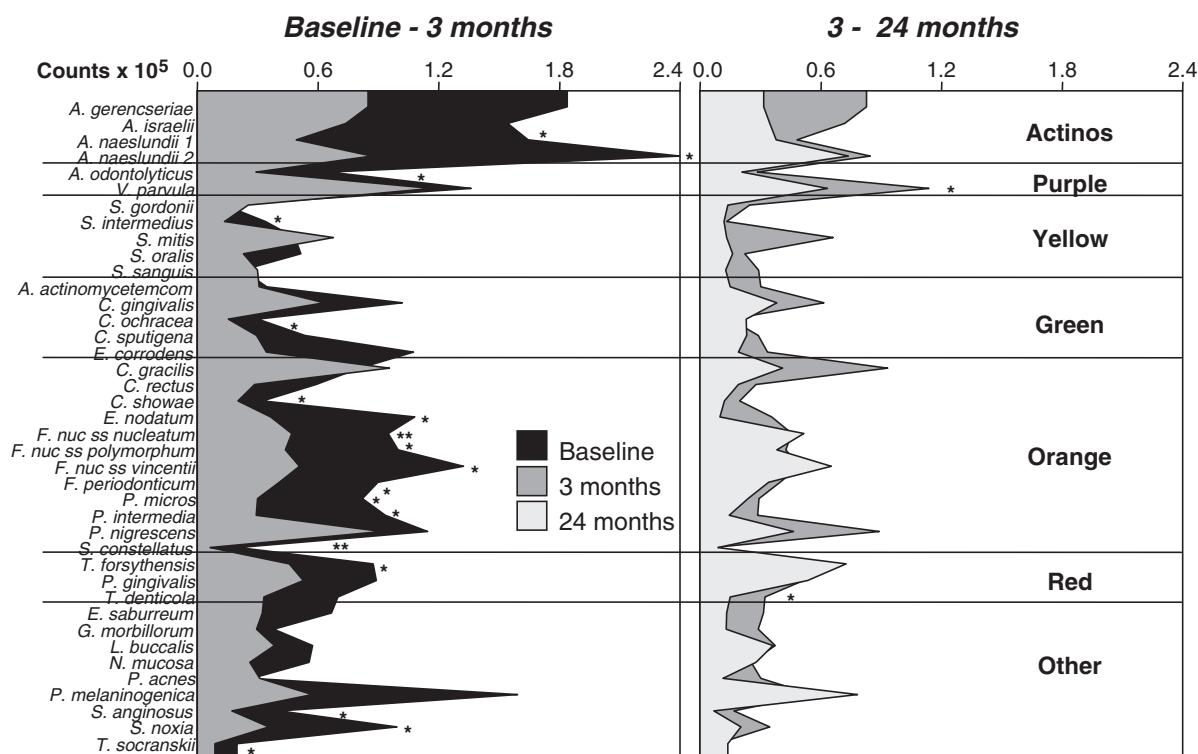


Fig. 5. Mean microbial profiles of 40 test species in 14 "refractory" subjects at baseline and 3 months (left panel) as well as 3 and 24 months (right panel). Mean counts for each species were computed in each subject and then averaged across subjects at each visit. Significance of differences between baseline and 3 months or between 3 and 24 months was sought using the Wilcoxon signed ranks test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The species were ordered and grouped according to the complexes described by Socransky et al. (1998).

mental factors such as cigarette smoking (MacFarlane et al. 1992). All of these hypotheses were presented with the understanding that proper home care procedures were being consistently employed by the "refractory" subject and that the "refractory" subject was, as far as could be determined, systemically healthy. In the current investigation the subjects were systemically healthy as determined by medical history. The subjects, however, did present with an average of 66% of surfaces harboring plaque at baseline. This score may be considered high but reflects the method of plaque assessment employed in which the detection of even the tiniest fleck of plaque on passing a probe over the tooth surface above the gingival margin is recorded as positive. However, visual inspection of the subjects in this study would suggest immaculate self-performed oral hygiene.

An interesting feature of the "refractory" subjects in this study was the lowered baseline counts of many of the species thought to be important in the initiation and progression of periodontal infections such as the red complex species, *T. forsythensis*, *P. gingivalis*

and *T. denticola* (Fig. 3). The multiple treatments that these "refractory" subjects had experienced prior to inclusion in the study may account for this observation. However, the lowered levels of periodontal pathogens brought about by previous therapy was not associated with an improvement in clinical status. Counts of other species were at comparable or higher levels in the refractory subjects prior to inclusion in the present investigation when compared with untreated periodontitis subjects. These included four of five *Actinomyces* species, *S. noxia*, *Propionibacterium acnes* and the "milleri" streptococci, *S. anginosus*, *S. constellatus* and *S. intermedius*. Members of this last group have been implicated in refractory forms of periodontitis by other investigators (Magnusson et al. 1991, Colombo et al. 1998a).

The reduced levels of the microbiota in the "refractory" subjects in this study were not sufficient to achieve periodontal stability. This may have been because of the presence of unusually virulent known periodontal pathogens, unknown pathogens or an inability of the host to cope with even

the lowered pathogen load. Nonetheless, whether the refractory state was because of unusual or virulent pathogens or a poor host response, the only feasible approach to treatment would be to lower the microbial load even further. The strategy employed was to combine therapies that were known to affect the subgingival microbiota and provide a beneficial clinical response. These therapies included SRP (Haffajee et al. 1997), systemically administered amoxicillin and metronidazole (van Winkelhoff et al. 1989, 1992, Pavicic et al. 1994, Winkel et al. 1997, 1998, Berglundh et al. 1998, Lopez & Gamonal 1998), locally delivered tetracycline (Kerry 1994, Vandekerckhove et al. 1997) and repeated professional supragingival plaque removal (McNabb et al. 1992, Magnusson et al. 1994, Ximenez-Fyvie et al. 2000). The therapies were chosen because they have different mechanisms of action on the microbiota and thus might have additive or even synergistic effects. SRP was employed to physically lower the biomass on the tooth surface and in the periodontal pocket. Local drug delivery was employed to directly diminish the pathogen load in

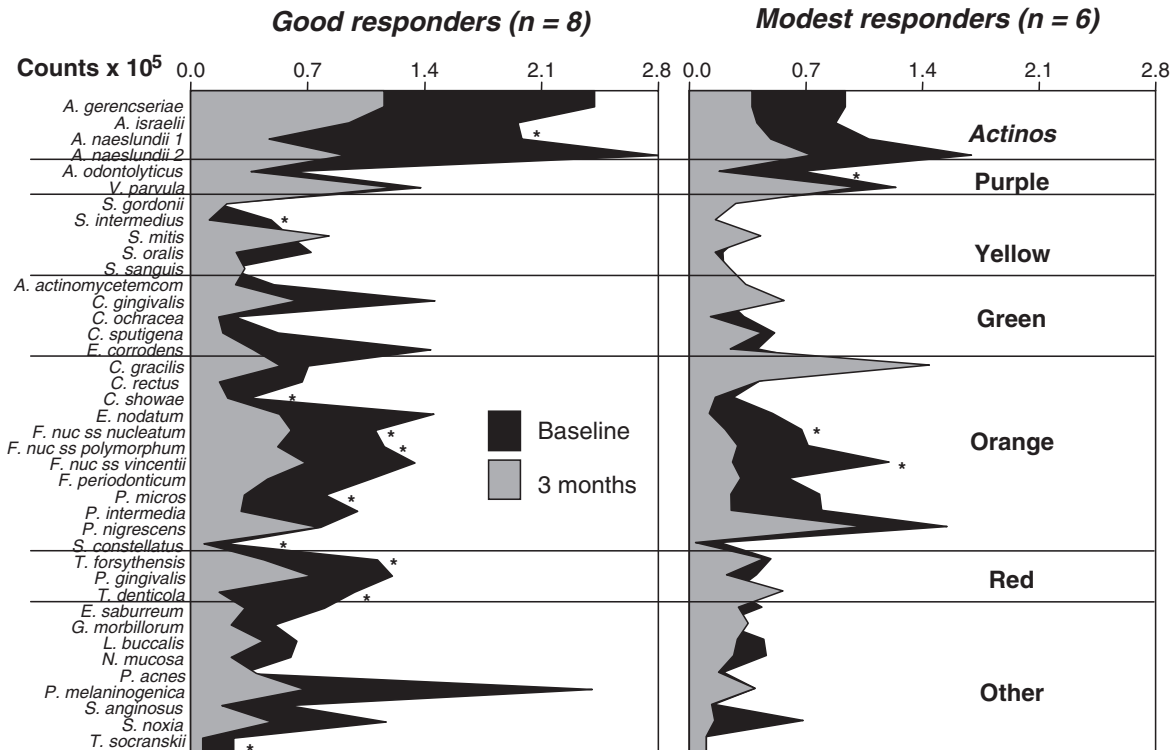


Fig. 6. Mean microbial profiles of 40 test species in eight good responding (left panel) and six modest responding subjects (right panel) at baseline and 3 months. Mean counts for each species were computed in each subject and then averaged across subjects in the two response groups at each visit. Significance of differences between baseline and 3 months in the two response groups was sought using the Wilcoxon signed ranks test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The species were ordered and grouped according to the complexes described by Socransky et al. (1998).

any residual pockets of ≥ 4 mm. Systemically administered amoxicillin plus metronidazole was employed to rapidly lower the level of pathogens throughout the oral cavity, particularly at sites with poor clinical access, and to attempt to control pathogenic species that might have entered the host's tissues. Repeated professional supragingival plaque removal was employed to help to control subgingival species by affecting the supragingival plaque (Ximenez-Fyvie et al. 2000) and for the beneficial effect that such therapy might have at shallower periodontal pockets that could not be readily instrumented (Haffajee et al. 2003).

The combined therapy was effective in rapidly lowering the levels of the species in the subgingival microbiota with the lowest levels being observed at the first post-therapy monitoring period at 3 months. The species at lowered levels included nine of 15 members of the orange complex, three *Actinomyces* species as well as the three species in the “*S. milleri*” group (Fig. 5). These species remained at lowered levels from 6 to 24 months. The reduction in the

microbial load translated into an excellent clinical outcome. Mean (\pm SEM) full-mouth pocket depth was reduced by 0.83 ± 0.13 mm for the 14 subjects evaluated at 24 months. Mean full-mouth attachment-level “gains” of 0.44 ± 0.12 mm were observed at the same time point.

While the overall data indicated clinical improvement, subjects varied in their response to therapy. For example, mean full-mouth pocket depth reduction ranged from 0.13 to 1.63 mm and full-mouth attachment-level measurements ranged from a mean loss of 0.08 to a “gain” of 1.27 mm at 24 months. Six subjects showed a modest response with mean attachment-level “gain” ranging from 0.13 to a loss of 0.08 mm, and eight subjects showed a good response ranging from 0.39 to 1.27 mm. There was a reduction in the microbiota in both groups at 3 months, which was particularly marked in the good response subjects (Fig. 6). In the good response group, the beneficial reductions achieved at 3 months were maintained to 24 months for most species and three species continued to

decline in mean counts. In the modest responding subjects, a number of species increased in mean counts from 3 to 24 months, in some instances returning to or exceeding baseline levels. The reason that one group of subjects showed greater reduction in mean counts than the other and was able to maintain these reductions is not known. The difference does not appear to be because of smoking status because there were comparable proportions of current and past smokers as well as mean pack years in the two response groups (five of eight good responders and three of six modest responders were current smokers with mean pack years of 29.7 and 24.0 in the two groups, respectively). Whatever the reason for the difference in the two response groups, failure to maintain a reduced microbiota, particularly for members of the orange and red complexes, appeared to lead to a worsening of the periodontal status.

Collins et al. (1993) also used a combined therapy to treat 30 subjects with refractory periodontitis based on the criteria that the subjects had received previous periodontal surgery, at

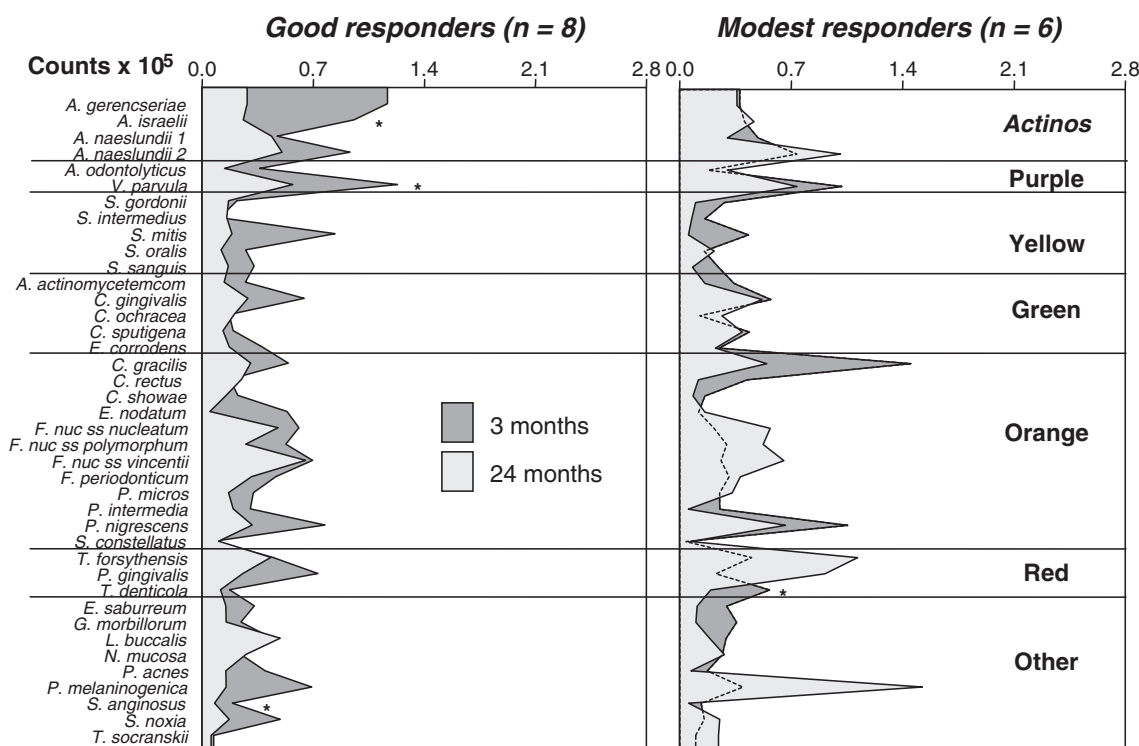


Fig. 7. Mean microbial profiles of 40 test species in eight good responding (left panel) and six modest responding subjects (right panel) at three and 24 months. Mean counts for each species were computed in each subject and then averaged across subjects in the two response groups at each visit. The dashed line in the right panel represents the 3-month data obscured by the 24-month data. Significance of differences between 3 and 24 months in the two groups was sought using the Wilcoxon signed ranks test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The species were ordered and grouped according to the complexes described by Socransky et al. (1998).

least one 10-day regimen of one or more systemically administered antibiotics, and a post-therapy history of progressive attachment loss or repeated abscess formation. In addition, all subjects exhibited excellent oral hygiene skills and minimal plaque accumulation at entry. Their therapy consisted of a 2-week regimen of systemically administered amoxicillin/clavulanate potassium, in conjunction with professional intrasulcular delivery of povidone iodine at six visits. The subjects also rinsed twice daily with 0.12% chlorhexidine. Consistent with the findings of the present investigation, there was a generally beneficial therapeutic outcome based on an overall mean attachment-level "gain" at 6 weeks. However, as in the present study, subjects differed in their response to therapy, with 26 of 30 subjects considered to exhibit a good therapeutic outcome, while four showed less benefit. The beneficial clinical changes in the best responding subjects were associated with a reduction in the prevalence of *P. gingivalis* at 6 weeks. Beneficial responses were also associated with reductions in "other black-pigmented *Bacteroides* species" and *T.*

forsythensis. Clinical data were available for 11 subjects at an average of 34 months post-therapy. These data indicated that, in general, the attachment-level improvement observed at 6 weeks was maintained out to an average of close to 3 years, although some subjects showed some mean attachment-level loss, while others showed continued improvement at the later time point.

The data of the present investigation suggest that "refractory periodontal disease" may exist, but that this condition may represent a state in which the clinician and the patient cannot lower the infectious burden below the level that can be tolerated by that host's innate and acquired resistance and environmental status. The present investigation and Collins et al. (1993) indicate that appropriate combinations of therapy can lower the already lowered infectious burden, brought about by previous therapy, to levels that are compatible with periodontal stability for extended periods of time in many, perhaps most, refractory subjects. These studies raise at least two questions. First, prior to treatment, how can subjects who will respond poorly to

"routine" periodontal therapy be distinguished from subjects who will respond well to therapy? Efforts at developing such diagnostic approaches are being carried out (Reinhardt et al. 1993, Jin et al. 1995, Colombo et al. 1999, Levine et al. 2002, Kurdowska et al. 2003). The second question is which therapy or therapies would be best to treat such individuals? It seems logical that anything that can be done to improve the host's resistance/environmental status should be encouraged. Such procedures might include convincing the individual to relinquish pernicious habits such as smoking or recreational drug use, reference to physicians to seek possible systemic conditions that might affect host resistance (e.g. diabetes or conditions that might compromise the immune system), guidance toward nutrition counseling or to approaches to lowering stress in his/her life. Perhaps more practical will be the development of techniques to lower pathogen load to the "extremely low" levels required by such individuals. The present study and that of Collins et al. (1993) used arbitrary combinations of therapy. The success of these combina-

tion therapies supports the promise that intense, sustained reduction of the infectious burden is a practical means to slowing or stopping "refractory periodontitis". What remains to be determined is which part(s) of these combinations are necessary or whether further or different adjuncts are warranted.

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Address:
A. D. Haffajee
Department of Periodontology
The Forsyth Institute
140 The Fenway
Boston, MA 02115
USA
E-mail: ahaffajee@forsyth.org

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