DR. RICARDO TELES (Orcid ID : 0000-0002-4216-2812)

PROF. WILLIAM V. GIANNOBILE (Orcid ID : 0000-0002-7102-9746)

Article type : Other

Patterns of Periodontal Disease Progression Based on Linear Mixed Models of Clinical Attachment Loss

Running title: Patterns of Periodontal Disease Progression

Ricardo Teles[1,2], Kevin Moss[3], John S. Preisser[4], Robert Genco[5], William V. Giannobile[6], Patricia Corby[7], Nathalia Garcia[8], Heather Jared[9], Gay Torresyap[2], Elida Salazar[2], Julie Moya[7], Cynthia Howard[7], Robert Schifferle[5], Karen L. Falkner[5], Jane Gillespie[8], Debra Dixon[8], MaryAnn Cugini[2].

[1]Department of Periodontics, Penn Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[2]Department of Applied Oral Sciences, Forsyth Institute, Cambridge, MA, USA.

[3]Department of Dental Ecology, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

[4]Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

[5]Department of Oral Biology, School of Dental Medicine, State University of New York at Buffalo, Buffalo, New York, USA.

[6]Department of Periodontics and Oral Medicine & Michigan Center for Oral Health Research, University of Michigan School of Dentistry, Ann Arbor, Michigan, USA.

[7]New York University College of Dentistry, Bluestone Center for Clinical Research, New York, NY, USA.

[8]Section of Periodontics, Department of Applied Dental Medicine, Southern Illinois University School of Dental Medicine, Alton, IL.

[9]Rho Inc., Contract Research Organization, Chapel Hill, NC, USA.

Address for correspondence (email can be published):

Ricardo Teles
Department of Periodontics
Penn Dental Medicine
University of Pennsylvania
240 South 40th Street
Philadelphia, PA 19104
Email: rteles@upenn.edu

ABSTRACT:

Aim: The goal of the present longitudinal cohort study was to examine patterns of periodontal disease progression at progressing sites and subjects defined based on linear mixed models (LMM) of clinical attachment loss (CAL). Methods: 113 periodontally healthy and 302 periodontitis subjects had their CAL calculated bi-monthly for 12 months. LMMs were fitted for each site and the predicted CAL levels used to categorize their progression state. Participants were grouped based on the number of progressing sites into unchanged, transitional and active subjects. Patterns of periodontal disease progression were explored using descriptive statistics. Results: Progression occurred primarily at molars (50% of progressing sites) and interproximal sites (72%), affected a higher proportion of deep than shallow sites (2.7% vs. 0.7%), and pocketing was the main mode of progression (49%). We found a low level of agreement (47%)

between the LMM and traditional approaches to determine progression such as change in CAL ≥3 mm. Fourteen percent of subjects were classified as active and among those 93% had periodontitis. The annual mean rate of progression for the progressing subjects was 0.35 mm/year. Conclusion: Progressing sites and subjects defined based on LMMs presented patterns of disease progression similar to those previously reported in the literature.

Key-words: Periodontal disease, Clinical attachment loss, Disease progression, Linear mixed models.

CLINICAL RELEVANCE:

Scientific rationale: Studies exploring patterns of periodontal disease progression in the literature relied on a limited number of visits for monitoring and on pairs of CAL measurements to define progression. Principal findings: The use of LMMs had a poor agreement with traditional approaches to define disease progression using thresholds of changes in CAL calculated using a pair of visits. However, patterns of disease progression were quite similar to those previously reported in the literature. Practical implications: Robust methods to define disease progression might result in more accurate assessments of the diagnostic and prognostic properties of clinical parameters and biomarkers.

Conflict of interest and source of funding statement: The authors have stated explicitly that there are no conflicts of interest in connection with this article. This study was supported by research grant DE021127-01 from the National Institute of Dental and Craniofacial Research.

INTRODUCTION:

Our understanding of periodontal disease progression comes from longitudinal studies examining the natural history of this condition. Longitudinal periodontal monitoring of untreated populations and subjects with access to different levels of oral care revealed common patterns of

disease progression (Loe et al., 1978a, Loe et al., 1986, Schatzle et al., 2003, Loe et al., 1978c, Loe et al., 1978b, Baelum et al., 1997, Heitz-Mayfield et al., 2003, Ismail et al., 1990, Lindhe et al., 1989a, Haffajee and Socransky, 1986, Socransky et al., 1984, Lindhe et al., 1983, Goodson et al., 1982, Machtei et al., 1997, Millen et al., 2014). Although annual mean rates of progression varied greatly, the picture that emerges is of a slow progressing disease, with significant progression being a sporadic event and the majority of progressing sites concentrating on a relatively small number of subjects (Lindhe et al., 1983, Schatzle et al., 2003, Baelum et al., 1997). Although loss of attachment can affect any tooth and site in the mouth, periodontal disease progression seems to occur primarily in pre-molars and molars (Schatzle et al., 2003, Thomson et al., 2006). Younger individuals seem to express loss of attachment through gingival recession, while pocketing becomes the main mode of disease progression as subjects get older (Schatzle et al., 2003). Pockets also seem to develop preferentially at interproximal sites, while the midbuccal and midlingual sites experience tissue destruction mainly through recession (Thomson et al., 2006, Schatzle et al., 2003). A tendency for the incidence of progression to increase with age has also been reported (Lindhe et al., 1989a, Haffajee et al., 1991), although this was not always the case (Baelum et al., 1997). Disease activity seems to affect a higher proportion of sites previously exposed to loss of attachment. However, because of the larger number of shallow sites in most subjects, progression affects a much higher number of sites without previous loss of attachment (Thomson et al., 2006, Lindhe et al., 1989b).

The vast majority of the literature reporting on periodontal disease progression has relied on pairs of visits to determine changes in clinical attachment loss (CAL). Disease progression was then defined based on increases in CAL above certain thresholds to account for 'error' in these measurements (Jeffcoat and Reddy, 1991, Lindhe et al., 1983, Goodson et al., 1982, Beck et al., 1994, Haffajee et al., 1983b, Aeppli et al., 1985, Deas et al., 1991). Even when measurements were obtained over several time points, reversals in CAL were, for the most, ignored (Lindhe et al., 1989a). Because of the uncertainties associated with a diagnosis of periodontal disease activity defined based on a single pair of measurements of CAL (Tonetti and Claffey, 2005, Corraini et al., 2013), it is possible that the patterns of disease progression previously reported were somewhat inaccurate. We have recently reported on the use of linear mixed models (LMM) to classify periodontal sites according to changes in clinical attachment loss over 12 months into different categories of disease progression (Teles et al., 2016). We

proposed that the categories of periodontal disease progression based on LMMs of CAL overcome several of the limitations from previous methodologies. A higher level of accuracy in designating a site as having undergone disease progression is paramount for the description of patterns of periodontal disease progression.

The data presented here were obtained from an ongoing study to search for biomarkers of periodontal disease progression. Since the previous report on the LMM approach to define disease progression, we have completed the clinical study and 427 participants were monitored for disease progression for 12 months. The goal of the present report was to describe the patterns of periodontal disease progression in this population, using the LMM approach to identify progressing sites and subjects. This is the largest number of subjects ever followed for 12 months without treatment with bi-monthly monitoring visits, allowing for a robust characterization of the patterns of periodontal disease progression.

MATERIAL & METHODS:

Study design:

The data were obtained as part of a prospective multi-center clinical study on biomarkers of periodontal disease progression (Teles et al., 2016). Participants were recruited between January 2012 and December 2014 at four clinical centers in the United States: The Forsyth Institute (Cambridge, MA), New York University College of Dentistry (New York, NY), the University at Buffalo, State University of New York (Buffalo, NY), and Southern Illinois University School of Dental Medicine (Alton, IL). Calibrated clinicians examined participants bi-monthly for 12 months to monitor changes in CAL measurements and determine disease progression. The Institutional Review Board from each center approved the study prior to its initiation.

Study population:

Inclusion and Exclusion Criteria:

The study had the following inclusion criteria: age ≥25 years; willingness to comply with study protocol; ≥20 natural teeth, excluding third molars; ≥12 of these teeth had to be pre-molars, first or second molars. Periodontitis subjects were stratified into mild periodontal loss

and severe periodontal loss. Participants with severe periodontal loss had at least 8 separate teeth with at least 1 site of pocket depth (PD) ≥5 mm and concomitant CAL ≥3 mm. Participants with mild periodontal loss had at least 4 teeth with at least 1 site of PD ≥5 mm and concomitant CAL ≥2 mm. Periodontitis subjects also had to present radiographic evidence of alveolar bone loss around at least 2 of the affected teeth. Periodontally healthy subjects met the following criteria: no radiographic evidence of alveolar bone loss, and all the teeth either had PD of ≤3 mm, irrespective of the attachment level; PD ≥4 mm with no CAL (except for the distal of the second molars); or, for distal of second molars, PD =4 mm with concomitant CAL ≤2 mm.

Exclusion criteria were: presence of orthodontic appliances; presence of intra-oral lesions at the time of screening; gross tooth decay; root fragments, pericoronitis, endo-perio lesions, or other dental abscesses; pregnancy or lactation; requirement for prophylactic antibiotics for dental procedures; periodontal or systemic antibiotic therapy in the previous 6 months; use of tobacco products within 1 year before the screening visit; any medical condition that might influence the course of periodontal disease or treatment; chronic use of nonsteroidal anti-inflammatory drugs; use of chronic systemic immunosuppressive agents; hypersensitivity to tetracyclines; and participation in a clinical study within the last 30 days. Only participants who had attended both the baseline and 12-month monitoring visit and had the same examiner throughout the monitoring phase were included. Participants were allowed to have only 1 missing visit. Further details can be obtained at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) under the identifier NCT01489839.

Clinical Examination:

Participants had periodontal parameters measured at up to 168 sites per subject (6 sites per tooth - mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual - for up to 28 teeth excluding third molars) including: probing depth (PD); measurement of distance from the cementoenamel junction (CEJ) to the free gingival margin (B measure) (in case of recession, a negative value was assigned); CAL (calculated by subtracting the B measure from the PD); presence or absence of plaque, gingival redness, BOP and suppuration. PD and the B measure were measured using calibrated North Carolina manual periodontal probes (PCPUNC 15 Hu-Friedy Co, Chicago, IL), rounding down to the nearest millimeter. At pre-molars, and at the first and second molars, PD and the B measure were measured twice. CAL was calculated for each

pass by the electronic data capturing (EDC) system. If the difference between the 2 measurements was ≥2 mm, the examiner was prompted by the EDC to obtain PD and the B measure a third time. The median CAL among the 2 or 3 passes was used for analysis.

Rescue Therapy:

Subjects with ≥6 sites with cumulative loss of attachment ≥2 mm from baseline during monitoring phase had their monitoring interrupted, and proceeded to treatment. Participants displaying ≥4 mm of CAL increase at a given site received periodontal rescue therapy at such sites and continued with monitoring. After the monitoring phase, periodontally healthy subjects received professional dental prophylaxis and exited the study, whereas participants with periodontal disease received non-surgical mechanical periodontal therapy.

Subjects and Sites Included in Analyses:

All participants who completed the 12-month monitoring phase, attended at least 6 out of 7 monitoring visits, and were examined by the same examiner in all such visits, had their data analyzed. Participants that had their monitoring interrupted due to rescue therapy were excluded. If a subject received rescue therapy in some but not all sites, data for such sites were removed from the analysis and the subject was otherwise retained in the analysis for any remaining sites.

Linear Mixed Models:

We applied linear mixed models (LMM) to predict subject-specific trends in CAL for each site and from which classifications of progression and regression were made, as previously described (Teles et al., 2016). Briefly, for each of the 168 tooth sites, a separate linear mixed effects model with a cubic polynomial for time (months) was fitted to quantify the course of progression within individuals. For additional details on the LMM employed, refer to Teles et al. 2016 and the online supplemental material.

We then developed a threshold for progression empirically based on the prediction standard errors from a second series of linear mixed models (again, one per site) fitted to ΔCAL_{it}, which is the change in CAL value from baseline to time=t (for t = 2, 4, 6, 8, 10 or 12 months) for subject i. These models are identical to the models described above, except that the outcome is ΔCAL_{ij}. The threshold for change was based on the 75^{th} percentile of the distribution of the standard errors for subject-specific predicted ΔCAL_{ij}. Sites were then classified as

progressing based on the predictions from the first series of linear mixed models using the threshold established from the second series. We grouped sites based on changes in pCAL (ΔpCAL) into: 1) regressing sites (ΔpCAL $<-2Q_{75}$); 2) stable sites ($-2Q_{75} \leq \Delta pCAL \leq 2Q_{75}$); 3) intermediate sites ($2Q_{75} < \Delta pCAL < 4Q_{75}$); and 4) progressing sites ($\Delta pCAL \geq 4Q_{75}$) (see Teles et al. 2016 for additional details).

Data Analyses:

All descriptive statistics, including mean periodontal clinical parameters and demographics, were computed using SAS® software and there was no imputation of missing data points. Mean periodontal clinical parameters were calculated for each subject and then across subjects in each clinical category separately for baseline and 12-month data. The mean change in CAL from baseline to 12 months was computed to estimate the 'annual mean rate of progression'. Subjects were also grouped into categories of progression such as unchanged (0 progressing sites), transitional (1-2 progressing sites) and active (≥3 progressing sites). Statistical significance of differences in the number of subjects in the three categories of progression across clinical groups (i.e., Healthy, Mild periodontal loss and Severe periodontal loss), was tested using the chi-square test. This analysis was also conducted grouping subjects based on CDC/AAP case definitions (Page & Eke 2007; Eke et al. 2012). Mean clinical parameters were then calculated for subjects in the three progression categories for baseline and 12-month data, including the 'annual mean rate of progression'. Significance of statistical differences across progression categories was determined using ANOVA. For certain analyses, periodontally healthy subjects were excluded from the progression categories. The mode of disease progression (i.e. pocketing, recession or both), was determined based on changes in PD and B measure from baseline to 12 months.

RESULTS:

Out of the 526 participants who attended a baseline visit, 53 subjects discontinue their participation after baseline due to different reasons, while 46 subjects had their monitoring interrupted due to rescue therapy (Figure 1). From the 427 participants who completed the 12-month monitoring, 12 were excluded because of a change in examiner or because they attended less than six monitoring visits, resulting in 415 participants in the final analysis. Among these

participants, 62 sites were excluded due to rescue therapy for a final number of 66,193 sites included in analyses. Periodontally healthy subjects tended to be younger, more likely to be female, and to have fewer missing teeth than subjects with mild or severe periodontal loss (Table 1). One can also observe that subjects in the healthy category presented less plaque, gingival redness, BOP and suppuration than the periodontitis groups. However, subjects classified as "periodontally healthy" were not necessarily periodontally intact and had an average CAL of 1.1 mm.

Safety Summary:

During the study, there were no unanticipated problems or serious adverse events reported. Out of 526 subjects who attended a baseline visit, 124 subjects required rescue therapy at the tooth site level but were able to remain in the monitoring phase of the study. Fifty-nine subjects transitioned to the treatment and maintenance phase to receive periodontal therapy based on the rescue therapy criteria for the study (see above). Twenty-eight teeth had to be extracted during the monitoring phase of the study. Reasons for extractions included: pain (5 teeth); dental caries (8 teeth); fracture (8 teeth); endodontic abscess (1 tooth); third molar extraction (2 teeth); periodontal reasons (2 teeth); and undetermined causes (2 teeth).

Linear mixed models for changes in CAL measurements:

From the linear mixed model for changes in CAL, the 75^{th} percentile for the standard errors of prediction was 0.242. Thus, the cut point for intermediate sites was selected as 0.474 mm, while the cutpoint for progressing sites was set at 0.948 mm.

Out of 66,193 sites examined, 86.2% were classified as stable and only 482 (0.7%) were classified as progressing based on the results from the LMMs (Table 2). Subjects with severe periodontal loss had three times higher proportion of progressing sites compared to periodontally healthy subjects. Fifty eight percent of the regressing sites were present in the severe periodontal loss group. At the subject level, 44% of participants presented at least one progressing site. Among periodontally healthy subjects, 22% had at least one progressing site compared to 53% for periodontitis subjects.

By computing the proportions of progressing sites across different tooth positions in the arch, it became apparent that the majority of progressing sites were located in the posterior

sextants (i.e. pre-molars and molars) (Fig. 2). Regarding site positions around the tooth, progression occurred more often at the mesiobuccal and distobuccal sites and least often at the midbuccal site (Table 3). When the distribution of progressing sites across different baseline pocket depths was calculated, one could observe that, although deep sites had a higher proportion of progressing sites (2.7% compared to 0.7% for shallow sites), progression affected a higher number of originally shallow sites (Table 4).

Pocketing was the main mechanism for disease progression, accounting for 48.7% of all progressing sites. Pure recession involved 21.7% of progressing sites, a combination of pocketing and recession affected 27.8%, while 1.2% of the progressing sites did not change in either PD or CAL from baseline to 12 months. Overall, periodontally healthy subjects had a higher proportion of sites progressing through recession (32%), compared to the mild periodontal loss (17%) and severe periodontal loss (22%) groups. When we examined the distribution of these three modes of loss of attachment across sites around the tooth, pure pocketing occurred mainly at interproximal sites. At the midbuccal site, disease progression occurred primarily through recession or a combination of pocketing and recession (Table S1).

We compared our categories of progression at the site level with traditional definitions of progression based on changes in CAL greater or equal to 2 or 3 mm. Only 257 sites (0.4%) had a change in CAL equal to or greater than 3 mm from baseline to 12 months, compared to 482 by LMM. Out of those, only 47% were classified as progressing based on the LMM, 44% as intermediate, and 8% as stable. One thousand eight hundred and ninety-six sites (2.9%) had a CAL change greater than or equal to 2 mm over 12 months. Nineteen percent of these sites were classified as progressing, 54% as intermediate, and 27% as stable.

We computed the distribution of participants into three categories of progression based on the number of progressing sites across clinical groups based on our case definitions and the AAP/CDC case definitions. Results indicated that there was an statistically higher proportion of active subjects in the periodontitis groups compared to healthy subjects, irrespective of the case definition used (Table 5).

The three categories of progressing subjects had comparable mean age, demographic parameters and number of missing teeth at baseline (Table S2). However, there were statistically significant differences in most clinical parameters, with the exception of suppuration. When

clinical parameters were compared across progression categories after the exclusion of periodontally healthy subjects, the majority of statistically significant differences disappeared (Table 5). However, the 12-month data revealed that most clinical parameters in the active group worsened during monitoring (Table 6). For instance, the change in mean number of sites with PD ≥4 mm was 5.4 for the active group compared to 0.1 and -6.6 for the transitional and unchanged groups, respectively. This resulted in statistically significant differences in most clinical parameters across categories of progression.

DISCUSSION:

The findings reported here were based on the largest cohort of subjects to be monitored for periodontal disease progression without therapy thus far. We used LMM of repeated measures of CAL to compensate for the high variability in this clinical parameter and achieve a more accurate diagnosis of disease progression (Teles et al. 2016). In the current study, we demonstrated that certain patterns of periodontal disease progression previously reported were also identifiable using this novel approach. For instance, advanced progression concentrated in a relatively small number of subjects, progression was a rare event, progression occurred primarily at interproximal sites, progression affected mainly molars, progression at interproximal surfaces occurred mainly through pocketing, while midbuccal sites had a higher prevalence of recession, and sites with deeper pockets tended to display a higher proportion of disease progression (Lindhe et al., 1989a, Haffajee et al., 1983a, Albandar et al., 1986, Loe et al., 1986, Papapanou et al., 1989, Schatzle et al., 2003, Baelum et al., 1997, Ismail et al., 1990).

Although the prevalence of progression was higher at sites with baseline PD >6 mm (2.7%) than at sites with PD <4 mm (0.7%), the number of shallow sites progressing (N = 387) was much higher than for deeper sites (N = 15), a finding in accord with others (Lindhe et al., 1989b, Thomson et al., 2006). This observation reaffirms the need for clinicians to focus of the disease process that afflicts their patients, rather than on the sequela of this process (i.e. deep pockets). The burden in terms of treatment needs imposed by disease progression is primarily the consequence of new diseased sites, rather than progression on previously affected sites.

Despite our best efforts to address hopeless teeth prior to enrollment, and a series of safety rules that triggered rescue therapy of monitored teeth and/or subjects, 28 teeth were lost

during the monitoring phase of the study. In addition, 24% of participants required rescue therapy due to having at least 1 site with an increase in CAL greater than 3 mm. Most extractions were associated with dental caries, fracture or pain associated with pulp pathology, and only two teeth were extracted due to deterioration of the periodontal condition. Although the number of teeth lost during the study was greater than desirable, the mean annual incidence of tooth loss per periodontitis subject (0.08/year) was similar to reports in the literature for subjects under periodontal maintenance (Teles et al., 2008, Checchi et al., 2002, Wood et al., 1989). That indicates that the tooth loss observed in our population of untreated subjects, compared favorably to studies on periodontitis patients receiving maintenance. This suggests that despite the lack of therapy, our participants were not exposed to an undue risk for tooth loss.

We calculated CAL based on measurements of PD and the distance from the cementoenamel junction (CEJ) to the free gingival margin. Although this method has been employed extensively in the periodontal literature, including large epidemiological surveys such as the National Health and Nutrition Examination Survey (NHANES) (Eke et al., 2015), some have argued that indirect measures of CAL can add to error because it requires two separate measurements (Corraini et al., 2013). However, direct measures of clinical CAL require considerable mental effort during the exam of sites where the CEJ is not exposed. For those areas, the examiner must mentally subtract the measured distance from the free gingival margin to the CEJ from the pocket depth (Corraini et al., 2013). In our experience, this method is not without inaccuracies and slows down the exam considerably. Further, the reproducibility obtained in CAL measures in our study compares favorably with the published literature, as can be ascertained by the high percentage of agreement and small stand deviations achieved with the two passes (online supporting information).

We arbitrarily grouped our subjects based on the number of progressing sites into ‘unchanged’, ‘transitional’ and ‘active’ subjects. By comparing the annual mean rate of progression for the subjects classified as active with historical controls (Schatzle et al., 2001, Schatzle et al., 2003, Baelum et al., 1997, Lindhe et al., 1989a, Ismail et al., 1990, Machtei et al., 1997), one can observe that we were able to select subjects with a high annual mean rate of progression. In the classic report on the natural history of periodontal disease in man by Löe et al. (Loe et al., 1986), the individuals identified as having rapid progression of periodontal disease had a mean annual rate varying from 0.10 to 1.0 mm/year. Among our subgroup of active

participants, the range was -0.66 to 1.2 mm/year and the mean was 0.35 mm/year. We interpreted these findings as indicative of the robustness of our criteria for selecting progressing sites and subjects.

We grouped subjects on three clinical groups based on their periodontal status. These criteria were established to accept a certain level of CAL as compatible with periodontal health, and to secure the recruitment of periodontitis subjects with a more severe and generalized pattern of disease, increasing our chances of observing disease progression. The drawback of establishing our own categories of disease was that the data cannot be promptly related to well-established standard case definitions, such as those proposed by the CDC and AAP (Page & Eke 2007; Eke et al. 2012). To partially address this concern, we determined the prevalence of unchanged, transitional and active subjects in both classifications. The results were reassuring given that the proportions of subjects in the three categories of progression were similar in each clinical group across the two classifications. For instance, the proportion of active subjects in the severe group was 28% and 23% for the CDC/AAP classification and our case definitions, respectively. These findings support the notion that our disease categories behaved similarly to those define based on CDC/AAP criteria, regarding susceptibility to progression.

When mean demographic and clinical parameters were compared across subject-level categories of progression, we observed that even at baseline, active subjects presented worse periodontal parameters. This would suggest that participants with more severe periodontal disease were more prone to progression. However, when only periodontitis subjects were examined, there were only minor differences across categories of progression at baseline. Due to a worsening of clinical parameters in the active group and an improvement in the unchanged group, by 12 months differences in clinical parameters were statistically significant across progressing groups. These findings support the notion that our definition of active subjects was capable of identifying individuals whose periodontal condition deteriorated during the one year of monitoring.

Previous studies on the progression of periodontal diseases reported pocketing as the primary mode of progression for individuals in the 45-49 age range; accounting for 73.3% of progressing sites, while pure recession and a combination of recession and pocketing affected 21.7% and 5.0% of progressing sites, respectively (Schatzle et al. 2003). In our study population,

48.7% of sites progressed through pocketing, 21.7% through recession, and 27.8% progressed through a combination of both mechanisms. Direct comparisons between our study and previous ones are compromised by the uniqueness of our approach to identify progressing sites using LMMs. Previous work has relied primarily on changes in CAL measurements obtained in a pair of visits to define progression. However, our results are in accord with the notion that pocketing is the primary mode of periodontal disease progression in adults. Further, our results confirmed previous studies indicating that buccal sites progress mainly through recession, while interproximal sites deteriorate primarily through pocketing (Schatzle et al., 2003). Also in accord with previous literature, periodontally healthy subjects had a higher proportion of sites progressing through recession compared with periodontitis individuals (Schatzle et al., 2003).

An important methodological difference between our study and the one by Schätzle et al. (Schatzle et al., 2003) is that they examined data from 2 sites (mesial, buccal) per tooth in the beginning and 4 sites (mesial, buccal distal, lingual, i.e. 50% buccal/lingual, 50% interproximal) later, while we measured 6 sites per tooth (33% buccal/lingual, 67% interproximal). Because interproximal sites progress mainly by pocketing, this difference in methodology may have impacted differences in the findings from the two studies.

Determining the mode of progression for progressing sites defined using LMMs also revealed certain limitations of this approach and a small percentage of progressing sites (1.7%) did not demonstrate increases in either PD or recession. Close inspection of the longitudinal profiles of observed and predicted CAL for these eight sites, revealed that outliers of CAL measurements resulted in the upward trend of change in the predicted CAL values. Another pattern observed suggested reversal of transitory changes in CAL measurements (data not shown).

In a previous paper, we illustrated how the LMM approach selected progression sites with a lower tendency to have increases in CAL reverse in subsequent visits, compared to sites selected based on changes in CAL on a pair of visits (Teles et al., 2016). When comparing the number of sites classified as progressing using the LMM to those using thresholds of changes in CAL measurements, for the higher threshold of ≥3 mm, only 47% of sites were classified as progressing based on the LMM. The lower threshold of 2 mm to define progression resulted in even higher proportions of mismatches to the LMM categories of progression. As highlighted in

our previous paper on the LMM approach, thus far, we have no means to determine with certainty which sites truly underwent progression. However, these results illustrate the discrepancies that would result from selecting different methods to define periodontal disease progression.

In summary, the use of LMM to characterize sites and subjects undergoing periodontal disease progression resulted in patterns of progression similar to those previously described in the literature. However, comparisons to traditional means to determine periodontal disease progression resulted in considerable discrepancies between which sites would be defined as progressing. Improvements in the diagnosis of sites and subjects that experienced periodontal disease progression might lead to more accurate assessments of the diagnostic and prognostic properties of clinical parameters and biomarkers. Future approaches using real-time assessment of disease activity based on biomarkers may improve the measurement accuracy of periodontal disease progression in at risk patients.

REFERENCES:

Aeppli, D. M., Boen, J. R. & Bandt, C. L. (1985) Measuring and interpreting increases in probing depth and attachment loss. *J Periodontol* **56,** 262-264. doi:10.1902/jop.1985.56.5.262.

Albandar, J. M., Rise, J., Gjermo, P. & Johansen, J. R. (1986) Radiographic quantification of alveolar bone level changes. A 2-year longitudinal study in man. *J Clin Periodontol* **13,** 195-200.

Baelum, V., Luan, W. M., Chen, X. & Fejerskov, O. (1997) A 10-year study of the progression of destructive periodontal disease in adult and elderly Chinese. *J Periodontol* **68,** 1033-1042. doi:10.1902/jop.1997.68.11.1033.

Beck, J. D., Koch, G. G. & Offenbacher, S. (1994) Attachment loss trends over 3 years in community-dwelling older adults. *J Periodontol* **65,** 737-743. doi:10.1902/jop.1994.65.8.737.

Checchi, L., Montevecchi, M., Gatto, M. R. & Trombelli, L. (2002) Retrospective study of tooth loss in 92 treated periodontal patients. *J Clin Periodontol* **29,** 651-656.

Corraini, P., Baelum, V. & Lopez, R. (2013) Reliability of direct and indirect clinical attachment level measurements. *J Clin Periodontol* **40,** 896-905. doi:10.1111/jcpe.12137.

Deas, D. E., Pasquali, L. A., Yuan, C. H. & Kornman, K. S. (1991) The relationship between probing attachment loss and computerized radiographic analysis in monitoring progression of periodontitis. *J Periodontol* **62,** 135-141. doi:10.1902/jop.1991.62.2.135.

Eke, P. I., Dye, B. A., Wei, L., Slade, G. D., Thornton-Evans, G. O., Borgnakke, W. S., Taylor, G. W., Page, R. C., Beck, J. D. & Genco, R. J. (2015) Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol* **86,** 611-622. doi:10.1902/jop.2015.140520.

Goodson, J. M., Tanner, A. C., Haffajee, A. D., Sornberger, G. C. & Socransky, S. S. (1982) Patterns of progression and regression of advanced destructive periodontal disease. *J Clin Periodontol* **9,** 472-481.

Haffajee, A. D. & Socransky, S. S. (1986) Attachment level changes in destructive periodontal diseases. *J Clin Periodontol* **13,** 461-475.

Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1983a) Clinical parameters as predictors of destructive periodontal disease activity. *J Clin Periodontol* **10,** 257-265.

Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1983b) Comparison of different data analyses for detecting changes in attachment level. *J Clin Periodontol* **10,** 298-310.

Haffajee, A. D., Socransky, S. S., Lindhe, J., Kent, R. L., Okamoto, H. & Yoneyama, T. (1991) Clinical risk indicators for periodontal attachment loss. *J Clin Periodontol* **18,** 117-125.

Heitz-Mayfield, L. J., Schatzle, M., Loe, H., Burgin, W., Anerud, A., Boysen, H. & Lang, N. P. (2003) Clinical course of chronic periodontitis. II. Incidence, characteristics and time of occurrence of the initial periodontal lesion. *J Clin Periodontol* **30,** 902-908.

Ismail, A. I., Morrison, E. C., Burt, B. A., Caffesse, R. G. & Kavanagh, M. T. (1990) Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Disease Study, 1959-87. *J Dent Res* **69,** 430-435.

Jeffcoat, M. K. & Reddy, M. S. (1991) Progression of probing attachment loss in adult periodontitis. *J Periodontol* **62,** 185-189.

Lindhe, J., Haffajee, A. D. & Socransky, S. S. (1983) Progression of periodontal disease in adult subjects in the absence of periodontal therapy. *J Clin Periodontol* **10,** 433-442.

Lindhe, J., Okamoto, H., Yoneyama, T., Haffajee, A. & Socransky, S. S. (1989a) Longitudinal changes in periodontal disease in untreated subjects. *J Clin Periodontol* **16,** 662-670.

Lindhe, J., Okamoto, H., Yoneyama, T., Haffajee, A. & Socransky, S. S. (1989b) Periodontal loser sites in untreated adult subjects. *J Clin Periodontol* **16,** 671-678.

Loe, H., Anerud, A., Boysen, H. & Morrison, E. (1986) Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. *J Clin Periodontol* **13,** 431-445.

Loe, H., Anerud, A., Boysen, H. & Smith, M. (1978a) The natural history of periodontal disease in man. Study design and baseline data. *J Periodontal Res* **13,** 550-562.

Loe, H., Anerud, A., Boysen, H. & Smith, M. (1978b) The natural history of periodontal disease in man. The rate of periodontal destruction before 40 years of age. *J Periodontol* **49,** 607-620. doi:10.1902/jop.1978.49.12.607.

Loe, H., Anerud, A., Boysen, H. & Smith, M. (1978c) The natural history of periodontal disease in man. Tooth mortality rates before 40 years of age. *J Periodontal Res* **13,** 563-572.

Machtei, E. E., Dunford, R., Hausmann, E., Grossi, S. G., Powell, J., Cummins, D., Zambon, J. J. & Genco, R. J. (1997) Longitudinal study of prognostic factors in established periodontitis patients. *J Clin Periodontol* **24,** 102-109.

Millen, A. E., Andrews, C. A., LaMonte, M. J., Hovey, K. M., Swanson, M., Genco, R. J. & Wactawski-Wende, J. (2014) Vitamin D status and 5-year changes in periodontal disease measures among postmenopausal women: the Buffalo OsteoPerio Study. *J Periodontol* **85,** 1321-1332. doi:10.1902/jop.2014.130686.

Papapanou, P. N., Wennstrom, J. L. & Grondahl, K. (1989) A 10-year retrospective study of periodontal disease progression. *J Clin Periodontol* **16,** 403-411.

Schatzle, M., Land, N. P., Anerud, A., Boysen, H., Burgin, W. & Loe, H. (2001) The influence of margins of restorations of the periodontal tissues over 26 years. *J Clin Periodontol* **28,** 57-64.

Schatzle, M., Loe, H., Lang, N. P., Heitz-Mayfield, L. J., Burgin, W., Anerud, A. & Boysen, H. (2003) Clinical course of chronic periodontitis. III. Patterns, variations and risks of attachment loss. *J Clin Periodontol* **30,** 909-918.

Socransky, S. S., Haffajee, A. D., Goodson, J. M. & Lindhe, J. (1984) New concepts of destructive periodontal disease. *J Clin Periodontol* **11,** 21-32.

Teles, R., Benecha, H. K., Preisser, J. S., Moss, K., Starr, J. R., Corby, P., Genco, R., Garcia, N., Giannobile, W. V., Jared, H., Torresyap, G., Salazar, E., Moya, J., Howard, C., Schifferle, R., Falkner, K. L., Gillespie, J., Dixon, D. & Cugini, M. (2016) Modelling changes in clinical attachment loss to classify periodontal disease progression. *J Clin Periodontol* **43,** 426-434. doi:10.1111/jcpe.12539.

Teles, R. P., Patel, M., Socransky, S. S. & Haffajee, A. D. (2008) Disease progression in periodontally healthy and maintenance subjects. *J Periodontol* **79,** 784-794. doi:10.1902/jop.2008.070485.

Thomson, W. M., Broadbent, J. M., Poulton, R. & Beck, J. D. (2006) Changes in periodontal disease experience from 26 to 32 years of age in a birth cohort. *J Periodontol* **77,** 947-954. doi:10.1902/jop.2006.050319.

Tonetti, M. S. & Claffey, N. (2005) Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *J Clin Periodontol* **32 Suppl 6,** 210-213. doi:CPE822 [pii] 10.1111/j.1600-051X.2005.00822.x.

Wood, W. R., Greco, G. W. & McFall, W. T., Jr. (1989) Tooth loss in patients with moderate periodontitis after treatment and long-term maintenance care. *J Periodontol* **60,** 516-520. doi:10.1902/jop.1989.60.9.516.

TABLES:

Table 1 - Demographic and clinical parameters of study subjects in the three clinical categories: periodontally healthy subjects, subjects with mild periodontal loss and subjects with severe periodontal loss at baseline and 12 months.

	Clinical Groups			
Parameters	Healthy	Mild	Severe	Total
N of Subjects (%)	113 (27%)	144 (35%)	158 (38%)	415 (100%)
N of Sites (%)	18,501 (28%)	22,766 (34%)	24,926 (38%)	66,193 (100%)
№ of Male/Female	29/84	59/85	71/87	159/256
Age (years; mean ± SD)	37 ± 12	52 ± 12	50 ± 12	47 ± 13
№ of AA/C/Other/ND	22/65/24/2	26/94/14/10	58/85/10/5	106/244/48/17
№ of Missing Teeth (mean ± SD)	0.7 ± 1.3	1.6 ± 1.5	1.6 ± 1.7	1.3 ± 1.6
Baseline Data				
PD (mm; mean ± SD)	1.7 ± 0.3	2.3 ± 0.3	2.8 ±0.5	2.3 ± 0.6
CAL (mm; mean ± SD)	1.1 ± 0.5	2.1 ± 0.5	2.5 ± 0.7	2.0 ± 0.8
Percentage of sites per subject with:				
Plaque (mean ± SD)	51 ± 24	65 ± 21	72 ± 21	64 ± 23
Gingival redness (mean ± SD)	26 ± 22	51 ± 25	65 ± 24	49 ±28
Bleeding on probing (mean ± SD)	20 ± 20	37 ± 20	54 ±24	39 ±25
Suppuration (mean ± SD)	0.02 ± 0.14	0.02 ± 0.11	0.17 ± 0.59	0.08 ± 0.38
№ of sites/subject				
CAL <4mm (Median, IQR)	168 (162-168)	143 (129-154)	128 (107-145)	142 (126-158)
CAL 4-6mm (Median, IQR)	0 (0-0)	14 (7-21)	28 (17-39)	16 (1-29)
CAL >6mm (Median, IQR)	0 (0-0)	0 (0-2)	1 (0-5)	0 (0-1)
№ of sites/subject				
PD <4mm (Median, IQR)	168 (152-168)	141 (132-149)	123 (105-139)	144 (124-159)
PD 4-6mm (Median, IQR)	0 (0-0)	16 (12-23)	34 (22-44)	14 (2-30)
PD >6mm (Median, IQR)	0 (0-0)	0 (0-1)	1 (0-5)	0 (0-1)
12-month Data				
PD (mm; mean ± SD)	1.8 ± 0.3	2.4 ± 0.3	2.8 ± 0.5	2.4 ± 0.6
CAL (mm; mean ± SD)	1.2 ± 0.5	2.1 ±0.6	2.5 ± 0.7	2.0 ± 0.8
Percentage of sites per subject with:				

Plaque (mean ± SD)	51 ± 27	64 ± 27	71 ± 25	63 ± 27
Gingival redness (mean ± SD)	32 ± 22	53 ± 26	67 ± 25	53 ± 29
Bleeding on probing (mean ± SD)	20 ± 20	35 ± 20	51 ± 25	37 ± 25
Suppuration (mean ± SD)	0.03 ± 0.14	0.04 ± 0.19	0.13 ± 0.44	0.07 ± 0.30
№ of sites/subject				
CAL <4mm (Median, IQR)	168 (160-168)	144 (128-155)	127 (109-146)	146 (124-162)
CAL 4-6mm (Median, IQR)	0 (0-0)	12 (5-25)	24 (13-38)	11 (0-27)
CAL >6mm (Median, IQR)	0 (0-0)	0 (0-1)	1 (0-5)	0 (0-1)
№ of sites/subject				
PD <4mm (Median, IQR)	166 (159-168)	142 (131-150)	124 (105-139)	144 (124-159)
PD 4-6mm (Median, IQR)	0 (0-2)	15 (8-23)	31 (18-44)	14 (2-30)
PD >6mm (Median, IQR)	0 (0-0)	0 (0-1)	1 (0-4)	0 (0-1)
Delta Observed CAL (mm; mean ± SD)	0.11 ± 0.29	0.03 ± 0.40	-0.03 ± 0.39	0.03 ± 0.37
Delta Predicted CAL (mm; mean ± SD)	0.12 ± 0.14	0.03 ± 0.17	-0.01 ± 0.17	0.04 ± 0.17

AA, African American; C, Caucasian; ND, Not disclosed.

PD – probing depth

CAL – clinical attachment loss

IQR – inter-quartile range

Table 2 - Number and percentage of sites (in parenthesis) for each of the four categories of progression from baseline to month 12, with classifications based on linear mixed model predictions of CAL stratified in the three clinical groups.

	Progression Category									
Clinical Groups	Regressing		Stable		Intermediate		Progressing		Total	
Healthy (N = 113)	279	(0.4%)	16,728	(25.3%)	1,444	(2.2%)	50	(0.1%)	18,501	(28.0%)
Mild PD (N = 144)	1,393	(2.1%)	19,592	(29.6%)	1,580	(2.4%)	214	(0.3%)	22,779	(34.4%)
Severe PD (N = 158)	2,315	(3.5%)	20,717	(31.3%)	1,663	(2.5%)	218	(0.3%)	24,913	(37.6%)
Total (N = 415)	3,987	(6.0%)	57,037	(86.2%)	4,687	(7.1%)	482	(0.7%)	66,193	(100%)

Table 3 - Number and percentage of sites (in parenthesis) for each of the four categories of progression at each site position around the tooth.

Progression Category	Site Position											
	MB		B		DB		DL		L		ML	
Regression	680	(6%)	821	(7%)	805	(7%)	634	(6%)	478	(4%)	569	(5%)
Stable	9,282	(84%)	9,458	(86%)	9,310	(84%)	9,592	(87%)	9,876	(89%)	9,519	(86%)
Intermediate	973	(9%)	704	(6%)	819	(7%)	712	(6%)	616	(6%)	863	(8%)
Progression	102	(0.9%)	64	(0.6%)	97	(0.9%)	77	(0.7%)	68	(0.6%)	74	(0.7%)

MB – mesiobuccal; B – midbuccal; DB – distobuccal; DL – distolingual; L – midlingual; ML – mesiolingual.

Table 4 - Number and percentage of sites (in parenthesis) for each of the four categories of progression stratified according to baseline pocket depth category.

	Progression Category			
Baseline PD	Regressing	Stable	Intermediate	Progressing
<4 mm	2359 (4%)	50701 (88%)	4160 (7.2%)	387 (0.7%)
4-6 mm	1447 (18%)	6010 (75%)	492 (6%)	80 (1.0%)
>6 mm	181 (33%)	326 (58%)	35 (6%)	15 (2.7%)

Table 5 - Number and percentage of subjects (in parenthesis) for each of the three categories of progression stratified according to clinical groups based of ours and the CDC/AAP case definitions.

PD CLASS	Unchanged		Transitional		Active*	
Healthy	88	(78%)	17	(15%)	8	(7%)
Mild	69	(48%)	47	(33%)	28	(19%)
Severe	74	(47%)	48	(30%)	36	(23%)
CDC/AAP	Unchanged		Transitional		Active*	
Healthy	96	(77%)	18	(14%)	11	(9%)
Mild/Moderate	84	(53%)	51	(32%)	24	(15%)

Severe	51	(39%)	43	(33%)	37	(28%)

*p<0.0001 based on chi-square test.

Table 6 - Clinical parameters of periodontitis subjects in the three categories of progression: unchanged (no progressing sites), transitional (subjects with 1-2 progressing sites), and active (subjects with 3 or more progressing sites) at baseline and 12 months.

	Progression Group				ANOVA
Parameter	Unchanged	Transitional	Active	Total	p-value
N of Subjects (%)	143 (47%)	106 (35%)	53 (18%)	302 (100%)	
N of Sites (%)	22,666 (47%)	16,661 (35%)	8,387 (18%)	47,714 (100%)	
№ of Missing Teeth (mean ± SD)	1.5 ± 1.5	1.7 ± 1.8	1.5 ± 1.5	1.6 ± 1.6	0.73
Baseline Data					
PD (mm; mean ± SD)	2.6 ± 0.5	2.6 ± 0.4	2.6 ± 0.5	2.6 ± 0.5	0.82
CAL (mm; mean ± SD)	2.3 ± 0.7	2.3 ± 0.7	2.4 ± 0.7	2.3 ± 0.7	0.67
Percentage of sites per subject with:					
Plaque (mean ± SD)	67 ± 21	66 ± 23	77 ± 16	68 ± 21	0.006
Gingival redness (mean ± SD)	57 ± 25	57 ± 26	63 ± 24	58 ± 25	0.27
Bleeding on probing (mean ± SD)	46 ± 24	45 ± 22	48 ± 26	46 ± 24	0.78
Suppuration (mean ± SD)	0.11 ± 0.52	0.08 ± 0.27	0.10 ± 0.46	0.10 ± 0.45	0.81
№ of sites/subject					
CAL <4mm (Median, IQR)	140 (123-153)	135 (118-147)	129 (116-147)	137 (119-150)	0.26
CAL 4-6mm (Median, IQR)	17 (8-32)	22 (12-33)	22 (12-33)	20 (11-33)	0.43
CAL >6mm (Median, IQR)	0 (0-2)	1 (0-3)	1 (0-6)	0 (0-3)	0.08
№ of sites/subject					
PD <4mm (Median, IQR)	135 (121-147)	133 (117-142)	132 (115-145)	134 (118-146)	0.89
PD 4-6mm (Median, IQR)	22 (15-35)	24 (15-35)	21 (16-37)	23 (15-35)	0.90
PD >6mm (Median, IQR)	0 (0-1)	0 (0-3)	0 (0-4)	0 (0-2)	0.08
12-month Data					
PD (mm; mean ± SD)	2.5 ± 0.4	2.6 ± 0.4	2.8 ± 0.5	2.6 ± 0.5	<0.0001
CAL (mm; mean ± SD)	2.1 ± 0.6	2.4 ± 0.7	2.7 ± 0.7	2.3 ± 0.7	<0.0001
Percentage of sites per subject with:					
Plaque (mean ± SD)	70 ± 27	62 ± 25	72 ± 22	68 ± 26	0.03
Gingival redness (mean ± SD)	64 ± 28	55 ± 24	63 ± 25	61 ± 27	0.04
Bleeding on probing (mean ± SD)	43 ± 25	41 ± 21	49 ± 26	43 ± 24	0.21
Suppuration (mean ± SD)	0.03 ± 0.19	0.13 ± 0.37	0.18 ± 0.55	0.09 ± 0.35	0.81
№ of sites/subject					
CAL <4mm (Median, IQR)	147 (129-158)	134 (118-146)	118 (103-131)	137 (118-151)	<0.0001

CAL 4-6mm (Median, IQR)	11 (3-23)	23 (13-33)	32 (17-44)	19 (7-32)	<0.0001
CAL >6mm (Median, IQR)	0 (0-1)	0 (0-4)	2 (0-5)	0 (0-3)	<0.0001
№ of sites/subject					
PD <4mm (Median, IQR)	140 (123-151)	133 (114-144)	121 (101-134)	134 (116-148)	<0.0001
PD 4-6mm (Median, IQR)	17 80-34)	23 (14-36)	30 (18-46)	21 (12-38)	0.0005
PD >6mm (Median, IQR)	0 (0-1)	1 (0-3)	1 (0-5)	0 (0-2)	<0.0001
Delta Observed CAL (mm; mean ± SD)	-0.18 ± 0.37	0.09 ± 0.30	0.33 ± 0.40	0.00 ± 0.40	<0.0001
Delta Predicted CAL (mm; mean ± SD)	-0.08 ± 0.16	0.04 ± 0.13	0.17 ± 0.16	0.01 ± 0.17	<0.0001

PD – probing depth

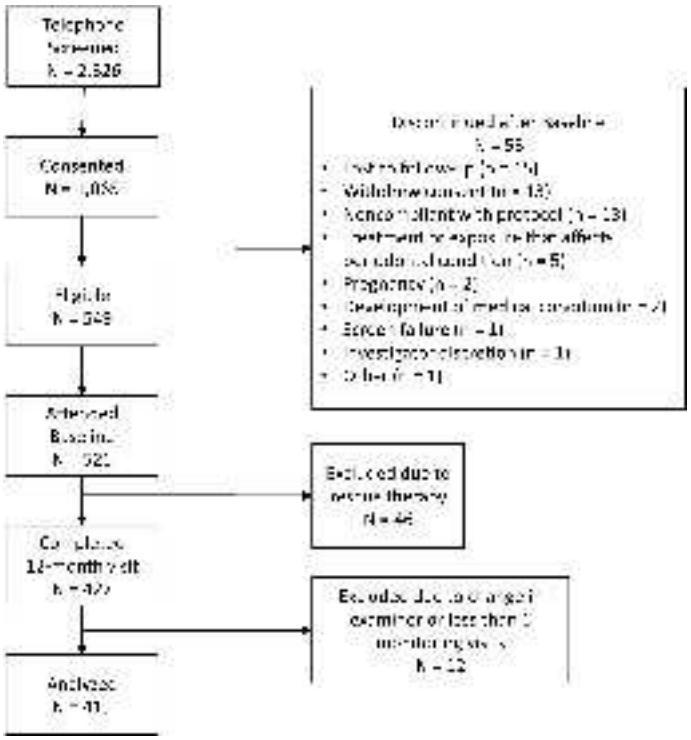
CAL – clinical attachment loss

IQR – inter-quartile range

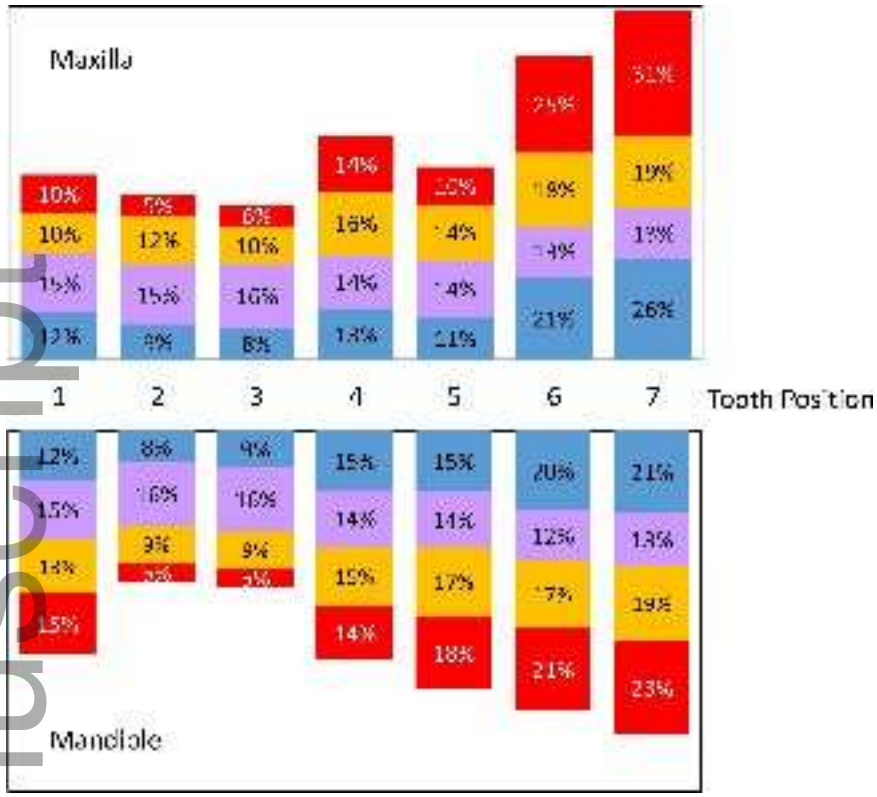
FIGURE LEGENDS:

Figure 1 – Flow chart of subject recruitment for the study: 2,526 subjects were telephone screened for this study; 1,065 subjects were enrolled (consented) in the study; 549 enrolled subjects were deemed eligible for the study after clinical screening; and 526 subjects attended a baseline visit. Of those, 53 discontinued due to different reasons, 46 subjects were moved to the treatment phase due to rescue therapy, and 427 subjects completed their 12-month visit. Twelve of these individuals were excluded due to change in the examiner during the monitoring phase, resulting in 415 subjects (113 periodontally healthy; 144 with mild periodontal loss and 158 with severe periodontal loss).

Figure 2 – Stack bar graph of frequency of occurrence of sites in different categories of progression: regressing (blue bars), stable (purple bars), intermediate (orange bars), and progressing (red bars) at different tooth positions in the upper and lower arches. Numbers correspond to different teeth in a quadrant, based on the FDI World Dental Federation notation.



jcpe_12827_f1.jpg



jcpe_12827_f2.jpg