Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2008; 35: 955–960 doi: 10.1111/j.1600-051X.2008.01315.x

Experimental gingivitis: reproducibility of plaque accumulation and gingival inflammation parameters in selected populations during a repeat trial

Leonardo Trombelli[1], Roberto Farina[1], Luigi Minenna[1], Alberto Carrieri[2], Chiara Scapoli[1,2] and Dimitris N. Tatakis[3]

[1]Research Center for the Study of Periodontal Diseases and [2]Department of Biology, University of Ferrara, Ferrara, Italy; [3]Section of Periodontology, College of Dentistry, The Ohio State University, Columbus, OH, USA

Trombelli L, Farina R, Minenna L, Carrieri A, Scapoli C, Tatakis DN. Experimental gingivitis: reproducibility of plaque accumulation and gingival inflammation parameters in selected populations during a repeat trial. J Clin Periodontol 2008; 35: 955–960. doi: 10.1111/j.1600-051X.2008.01315.x.

Abstract

Objectives: This study aimed to assess whether and to what extent the experimental gingivitis clinical parameters are reproducible within selected populations with different gingival inflammatory response (high or low) to plaque accumulation. In addition, the consistency in developing a high or low gingival inflammatory response within the selected populations was evaluated.

Material and Methods: Thirty-seven subjects previously identified as high (HR, $n = 20$) or low responders (LR, $n = 17$) during an experimental gingivitis trial (first trial) were enrolled in a ''repeat'' experimental gingivitis trial.

Results: No significant differences in plaque accumulation parameters and bleeding index values were detected between first and repeat trial for the 37 participants. Gingival index was higher during the repeat trial but behaved consistently in terms of the temporal changes in the course of both trials in both populations. Of the 17 LR participants, 10 manifested low susceptibility to inflammation after repeat trial. Among the 20 HR, 10 manifested high susceptibility to inflammation after repeat trial.

Conclusions: These results indicate that our experimental gingivitis model is reproducible to some extent in selected populations. The high reproducibility of plaque and, to a lesser extent, of inflammation parameters under the employed controlled conditions could be a valuable tool in gingivitis research.

Key words: gingivitis; periodontal diseases

Accepted for publication 31 July 2008

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This study was supported by the Ministry of Education, University and Research of Italy (grant ''ex 60% 2002–2004'', University of Ferrara), and by GABA International AG, Münchenstein, Switzerland.

Since the aetiologic role of dental bacterial plaque in gingivitis was definitively demonstrated 40 years ago (Löe et al. 1965, 1967, Theilade et al. 1966), significant inter-individual differences in onset and severity of the gingival inflammatory response to plaque were originally ascribed to quantitative and/or qualitative plaque differences (Löe et al. 1965, Theilade et al. 1966). A review of subsequent experimental gingivitis literature, however, indicates that susceptibility to plaque-induced gingivitis may differ significantly among subjects, in the absence of plaque differences (Tatakis & Trombelli 2004).

The reported significant differences in gingival inflammatory response under quantitatively and/or qualitatively almost identical plaque accumulation

(Abbas et al. 1986, Lie et al. 1995, Trombelli et al. 2004a, b, c) suggest that the gingival response to plaque accumulation may be an individual trait (Abbas et al. 1986, Tatakis & Trombelli 2004), dependent on host-related factors, possibly genetic in origin (Tatakis & Trombelli 2004, Scapoli et al. 2005, 2007). An immediate implication of such a tenet is that a subject's gingival inflammatory response will be consistently high or low relative to plaque exposure levels, while some studies reported that a percentage of repeatedly tested participants show consistently high or low inflammatory response to de novo plaque accumulation (Watts 1978, van der Weijden et al. 1994), others showed that there is little, if any, agreement between individual responses in repeated trials (Shearer et al. 2005).

The aim of the present study was to assess whether and to what extent the experimental gingivitis clinical parameters, i.e., plaque accumulation and gingival inflammatory response levels, are reproducible at different times within selected populations, i.e., two groups previously identified as having different gingival inflammatory response (high or low) to plaque accumulation. In addition, the consistency in developing a high or low gingival inflammatory response within the selected populations was evaluated.

Material and Methods

Experimental design and study population

The study protocol was approved by the Research Ethics Committee, University of Ferrara, and all participants provided written informed consent. After a first randomized split-mouth localized experimental gingivitis clinical trial (first trial), conducted from October 2000 to November 2001, two sub-populations were identified, respectively, defined as high responders (HR, $n = 24$) and low responders (LR, $n = 24$), and characterized by significantly different severity of gingivitis to similar amounts of plaque deposits (Trombelli et al. 2004c). On January 2002, we recalled all HR and LR individuals to verify their availability and eligibility for a second trial (repeat trial).

Volunteers underwent the repeat trial between April and November 2002. The overall experimental design was identical to the first trial (Trombelli et al. 2004c). Briefly, after a period of professional and supervised tooth cleaning (starting on day 14), one maxillary quadrant was randomly assigned as ''experimental'' (experimental gingivitis) and the contra-lateral as ''control'' (oral hygiene continuation) according to a computer-generated randomization list. Three teeth were used in each quadrant: lateral incisor, first bicuspid and first molar, with clinical parameters evaluated on two sites (buccal, mesio-buccal) per tooth. Examiners were kept unaware of randomization sequence and masked as to quadrant (control or test) and subject (LR or HR, as categorized following original trial) status. Yet, differences in plaque accumulation and severity of gingival inflammation occurred in test and control quadrants throughout the experimental phase may have compromised the blindness of the examiners with respect to quadrant allocation.

After 21 days of oral hygiene withdrawal, all the subjects were re-evaluated, given oral hygiene instructions, and re-evaluated again after 7 days of self-performed plaque control in both experimental and control quadrants (day 28), when professional tooth cleaning was provided as needed.

Clinical parameters

The following parameters, detailed previously (Trombelli et al. 2004c), were obtained in the order listed: gingival index (GI), plaque index (PlI), angulated bleeding score (AngBS) and the derived parameter cumulative plaque exposure (CPE) (= area under the curve of subject-specific PlI over a specific time period) was calculated. All the parameters were recorded on days 14 (baseline), 0, 7, 14 and 21 by two trained and calibrated examiners with good to excellent intra- and inter-examiner agreement (Trombelli et al. 2004c). Gingival crevicular fluid (GCF) was collected and dental plaque was sampled; details on GCF and plaque related methodologies and results will be reported elsewhere.

Statistical analysis

Plaque accumulation and inflammatory gingival response (entire study population)

The subject was the statistical unit. For each parameter, recordings from the six selected sites (three selected teeth, two sites per tooth) per quadrant were averaged to obtain the subject mean value for each quadrant. Therefore, for each parameter at each observational period, the subject was represented by a single test and a single control value. Data were expressed by median and interquartile range (IR) for non-parametric variables or mean $\pm$ standard deviation (SD) for parametric variables.

To test the effect of ''time'', ''quadrant'' and ''experimental phase'' (first *versus* repeat trial) on response variables, a three-way ANOVA for repeated measures for parametric variables (PlI, CPE) was used. For non-parametric variables (GI, AngBS), Friedman's test and post hoc comparisons were performed to explore intra- and inter-quadrant as well as inter-phase differences. The level of significance was set at 5%.

Level of consistency in gingival inflammatory response to plaque in HR/LR subjects

The assumption was that during the repeat trial, LR subjects would demonstrate lower severity of gingival inflammation to similar amount of plaque accumulation when compared with HR. To assess consistency of the gingival response to plaque in HR/LR subjects, the following approach was used.

First, demographic data, plaque and gingival inflammation parameters of LR and HR, as assessed at the repeat trial, were compared, by using (two test for dichotomous variables, and unpaired *t*-test and Mann–Whitney *U* test for parametric and non-parametric variables, respectively. McNemar's χ^2 test was used to compare the frequency distribution of subjects in the different groups.

To assess the individual variability in gingival inflammatory response to plaque for the repeat trial population, the ratio between GI and CPE was chosen as the primary outcome variable. As (log e GI)/(log e CPE) – day 21 in test quadrant was normally distributed, we used the test quadrant (log e GI)/(log e CPE) – day 21 mean value (= – 0.0455) to identify two subsets of individuals: one subset ($n = 20$) with a day 21 (log e GI)/(log e CPE) (test quadrant) value below the mean, i.e., with low susceptibility to plaque-induced gingival inflammation (defined as LS subjects); and one subset ($n = 17$) with a day 21 (log e GI)/(log e CPE) (test quadrant) value above the mean, i.e., with a high susceptibility (defined

as HS). These two subsets were characterized by significantly different gingival inflammation (day 21 test quadrant GI: 0.72 ± 0.12 *versus* 1.10 ± 0.15 in LS and HS, respectively; $p < 0.000$), but similar plaque deposits (day 21 test quadrant PlI: 1.81 ± 0.25 *versus* 1.75 ± 0.46 in LS and HS, respectively; $p = 0.602$) and plaque exposure (day 21 test quadrant CPE: 27.36 ± 3.35 *versus* 27.55 ± 5.49 in LS and HS, respectively; $p = 0.895$).

Finally, the prevalence of HR and LR in the two subsets of individuals, LS and HS, was calculated and analysed.

Results

Study population

Of the originally identified 48 HR/LR subjects (Trombelli et al. 2004c), 37 (20 males and 17 females; mean age: 23.7 ± 1.8 years) volunteered for the repeat trial. Seventeen subjects belonged to the LR group (nine males and eight females; mean age: 23.4 ± 2.0 years) and 20 belonged to the HR group (11 males and nine females; mean age: 24.0 ± 1.6 years). All the 37 subjects completed the repeat trial complying with study instructions. In the repeat trial, no significant differences were found in age and gender between LR and HR groups.

There were no changes in the study population recruited in the repeat trial with respect to the first trial in terms of smoking habits, recall frequency, dietary habits, medications or medical status.

Plaque accumulation and gingival response in the entire study population ($n = 37$) during repeat trial

ANOVA revealed a statistically significant effect of ''time'' and ''quadrant'' on both PlI ($p < 0.0001$ for both factors) and CPE ($p < 0.0001$ for both factors). In test quadrants, PlI was 0.39 ± 0.30 at day 0, 1.29 ± 0.29 at day 7, 1.53 ± 0.28 at day 14 and 1.78 ± 0.36 at day 21. Test quadrant CPE was 5.88 ± 1.62 at day 7, 15.76 ± 2.96 at day 14 and 27.45 ± 4.40 at day 21. Control quadrant PlI and CPE remained similar to baseline, and were significantly different from test-quadrant parameters at days 7, 14 and 21 ($p < 0.0001$ at any time for both PlI and CPE).

Statistically significant increases in GI ($p < 0.0001$) and AngBS ($p < 0.0001$) were observed in test quadrants over the course of the trial. GI was 0.33 (IR: 0.17–0.50) at day 7, 0.67 (IR: 0.50–0.83) at day 14 and 0.83 (IR: 0.67–1.00) at day 21. AngBS was 0.00 (IR: 0.00–0.17) at day 7, 0.17 (IR: 0.00–0.33) at day 14 and 0.33 (IR: 0.17–0.50) at day 21. Control quadrant GI and AngBS remained close to zero throughout the trial, and were significantly different from test-quadrant parameters at days 7, 14 and 21 ($p < 0.0001$ at any time for both AngBS and GI).

Comparison of plaque accumulation and gingival inflammation parameters as assessed during first trial *versus* repeat trial, by subgroup

Statistical analysis of the respective data from the first trial in comparison to the data from the present (repeat) trial for the 37 participants revealed no differences in either LR or HR subjects in PlI (Table 1), CPE (Table 2) and AngBS (Table 3) in test quadrants for each observation interval, with the exception of day 21 AngBS values in HR. However, GI during repeat trial was significantly higher compared with first trial for both LR and HR at each observation interval, with the exception of the day 21 HR values (Table 4).

Comparison between HR and LR during first and repeat trial

During the first trial, the test quadrant PlI, CPE and AngBS of the 20 HR subjects participating in the repeat trial were not different, at any time point, from the respective parameters of the 17 LR subjects participating in the repeat trial ($p > 0.05$). Similarly, during the repeat trial, the two subgroups showed no differences in PlI, CPE and AngBS ($p > 0.05$).

During the first trial, HR had significantly higher GI levels in test quadrant compared with LR at day 7 (0.17 *versus* 0.00, $p = 0.020$), day 14 (0.50 *versus* 0.17, $p = 0.003$) and day 21 (0.83 *versus* 0.50, $p = 0.003$, respectively). No GI differences were detected between LR and HR in control quadrants at each observation interval of the first trial ($p > 0.05$). During the repeat trial, GI levels were not different between LR and HR in either test or control quadrants ($p > 0.05$) at each observation interval.

Table 1. Descriptive statistics and comparisons among mean (± standard deviation, SD) levels of plaque index measured in low responders and high responders subjects at first and repeat trials in test quadrant

	n	First trial mean ± SD	Repeat trial mean ± SD	*p*-value
Low responders				
Day 0	17	0.36 ± 0.237	0.34 ± 0.260	1.000
Day 7	17	1.30 ± 0.214	1.29 ± 0.326	1.000
Day 14	17	1.59 ± 0.264	1.53 ± 0.286	1.000
Day 21	17	1.70 ± 0.271	1.79 ± 0.315	0.998
High responders				
Day 0	20	0.43 ± 0.317	0.44 ± 0.334	1.000
Day 7	20	1.26 ± 0.410	1.31 ± 0.261	0.999
Day 14	20	1.57 ± 0.335	1.53 ± 0.297	0.999
Day 21	20	1.74 ± 0.331	1.77 ± 0.402	1.000

Table 2. Descriptive statistics and comparisons among mean levels (± standard deviation, SD) of cumulative plaque exposure measured in low responders and high responders subjects at first and repeat trials in test quadrant

	n	First trial mean ± SD	Repeat trial mean ± SD	*p*-value
Low responders				
Day 0	17	0.36 ± 0.237	0.34 ± 0.260	1.000
Day 7	17	5.83 ± 0.945	5.73 ± 1.476	1.000
Day 14	17	15.96 ± 2.113	15.65 ± 3.282	1.000
Day 21	17	27.45 ± 3.430	27.31 ± 4.727	1.000
High responders				
Day 0	20	0.43 ± 0.317	0.44 ± 0.334	1.000
Day 7	20	5.92 ± 2.101	6.12 ± 1.698	1.000
Day 14	20	15.84 ± 4.078	16.04 ± 2.700	1.000
Day 21	20	27.45 ± 5.859	27.56 ± 4.219	1.000

Table 3. Descriptive statistics and comparisons among median (interquartile range, IR) levels of angulated bleeding score measured in low responders and high responders subjects at first and repeat trials in test quadrant

	n	First trial median (IR)	Repeat trial median (IR)	*p*-value
Low responders				
Day 0	17	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.000
Day 7	17	0.0 (0.0–0.0)	0.0 (0.0–0.17)	0.999
Day 14	17	0.33 (0.0–0.50)	0.33 (0.0–0.50)	0.999
Day 21	17	0.33 (0.33–0.67)	0.33 (0.17–0.50)	0.999
High responders				
Day 0	20	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.000
Day 7	20	0.17 (0.0–0.25)	0.0 (0.0–0.17)	1.000
Day 14	20	0.17 (0.0–0.42)	0.17 (0.0–0.33)	0.834
Day 21	20	0.67 (0.42–1.0)	0.33 (0.17–0.50)	0.002

Table 4. Descriptive statistics and comparisons among median (interquartile range, IR) levels of gingival index measured in low responders and high responders subjects at 1st and repeat trials in test quadrant

	n	First trial median (IR)	Repeat trial median (IR)	*p*-value
Low responders				
Day 0	17	0.0 (0.0–0.0)	0.0 (0.0–0.0)	–
Day 7	17	0.0 (0.0–0.0)	0.33 (0.17–0.50)	<0.001
Day 14	17	0.17 (0.0–0.33)	0.67 (0.33–0.83)	<0.001
Day 21	17	0.50 (0.33–0.50)	0.83 (0.83–1.0)	<0.001
High responders				
Day 0	20	0.0 (0.0–0.0)	0.0 (0.0–0.0)	–
Day 7	20	0.17 (0.0–0.33)	0.50 (0.33–0.58)	<0.001
Day 14	20	0.50 (0.33–0.67)	0.67 (0.58–0.75)	0.03
Day 21	20	0.83 (0.58–0.92)	0.92 (0.67–1.0)	0.38

Consistency in gingival inflammatory response to plaque accumulation as assessed during first and repeat trial

Of the 17 LR participants, 10 were present in the LS subset and seven in the HS. Among the 20 HR, 10 were present in the HS subset and 10 in the LS. Distribution of LR and HR in the LS/HS subsets was not statistically significant.

When clinical parameters related to plaque (PlI, CPE) and gingival inflammation (GI, AngBS) were compared in the 10 LR subjects of the LS subset (LR-LS) and the 10 HR subjects of the HS subset (HR-HS), no differences were found with respect to test quadrant PlI, CPE and AngBS (data not shown). However, test quadrant GI showed a statistically significant difference between the groups at days 14, 7 and 21 (Table 5).

At day 14, GI/PlI ratio, as calculated for both test and control quadrants combined, was 0.27 ± 0.3 in LR-LS subjects and 0.95 ± 1.12 in HR-HS subjects, the difference being statistically significant ($p = 0.001$).

Discussion

The results of the present study indicate that during a repeated experimental gingivitis trial, under well-controlled experimental conditions (inclusion of control quadrants, use of stents to avoid inadvertent plaque removal at test quadrants, vitamin C supplementation to reduce environmental effects of potential differences in vitamin C intake), the model is to some extent reproducible in selected populations. Specifically, the plaque accumulation parameters and the bleeding index values showed no differences, despite the time separating the trials and regardless of the group examined. GI, although it was higher during the repeat trial compared with the first trial, behaved consistently in terms of the temporal changes in the course of both trials in both populations. The results of the present study also indicate that a proportion of the subjects (ranging from 50% to 59%), regardless of whether they were initially categorized as HR or LR, had a, respectively, consistent high or low gingival inflammatory response to plaque accumulation during the repeat trial.

An important modification of the present model has been the use of partial-mouth experimentation (one quadrant, three teeth, six sites) as opposed to total oral hygiene abstinence. Although the ''partial-mouth'' assessment may have potentially limited the available information on the extent and rate of plaque accumulation and gingival inflammation compared with the ''full-mouth'' assessment, this modification made the experimental gingivitis model easier to accept by the prospective participants, therefore more feasible from a practical perspective. It also lessened the probability of adverse effects by limiting the number of teeth on which plaque was allowed to accumulate, rendering the model preferable from an ethical perspective (Trombelli et al. 2004b). Previous reports included use of two quadrants (Deinzer et al. 1999), one quadrant (Putt et al. 1993, van der Weijden et al. 1994) and any number of contiguous teeth (Bosman & Powell 1977, Matheny et al. 1993, Daly & Highfield 1996), extending over one (Bosman & Powell 1977, Daly & Highfield 1996) or two quadrants (Matheny et al. 1993). The results of partial-mouth (''localized'') experimental gingivitis studies have always been similar to the results of full-mouth studies, making the

Table 5. Descriptive statistics and comparisons among median (interquartile range, IR) levels of gingival index measured in low responders-low susceptible (LR-LS) and high responders-high susceptible (HR-HS) subjects at different time in test quadrant

Test quadrant	*n*	LR-LS	*n*	HR-HS	
		median (IR)		median (IR)	Mann–Whitney test (*p*-value)
Day 14	10	0.17 (0.0–0.33)	10	0.58 (0.33–0.67)	0.005
Day 0	10	0.0 (0.0–0.0)	10	0.0 (0.0–0.0)	1.000
Day 7	10	0.17 (0.17–0.33)	10	0.50 (0.50–0.67)	0.002
Day 14	10	0.42 (0.33–0.83)	10	0.67 (0.67–0.83)	0.093
Day 21	10	0.83 (0.67–0.83)	10	1.00 (1.00–1.17)	<0.001

''localized'' assessment equivalent to the original full-mouth one.

The comparison of the clinical parameters between the first and the repeat trial, whether within the 17 LR subject group or the 20 HR subject group, revealed that there were no differences in PlI or the derived parameter CPE. This result regarding PlI is consistent with the findings of van der Weijden et al. (1994); in a study of similar design that included 25 subjects, they reported no differences in PlI, either at baseline or at day 21, between a first and a repeat experimental gingivitis trial, performed 6 months apart. Collectively, the PlI results from the two studies suggest that, under well-controlled conditions, the experimental gingivitis model can be highly reproducible in terms of the quantitative aspects of supragingival plaque accumulation.

All comparisons between first and repeat trial, whether within the LR group or the HR group, revealed no differences in AngBS (with the sole exception of the day 21 HR group values). This result is also consistent with the respective findings of van der Weijden et al. (1994); the day 21 bleeding index values were not different between their first and repeat trial. Taken together, the results of the two studies suggest that, under well-controlled conditions, the experimental gingivitis model can be reproducible in terms of the bleeding tendency aspect of the gingival inflammatory response.

The consistency between the results of the present study and the study of van der Weijden et al. (1994), regarding reproducibility of PlI and bleeding scores during repeat trials, contrasts with the lack of reproducibility reported by Shearer et al. (2005). Among the several methodological differences that could account for the disparate results between these studies, one that stands out is the choice of posterior mandibular teeth by Shearer et al. (2005) and maxillary teeth by van der Weijden et al. (1994) and the present study. However, whether and to what extent the selection of specific tooth types may affect either the severity of the experimentally induced gingival inflammation or the clinical parameters related to plaque accumulation or gingival inflammation remains still undetermined.

The present study found that during the repeat trial, both LR and HR subjects had significantly greater GI levels, compared with the first trial (with the sole exception of the day 21 HR values). As far as we could ascertain, there are no other studies involving repeated experimental gingivitis trials that provide data comparisons for GI. One explanation for this inconsistent GI results between the first and repeat trials may involve the low level of reproducibility for GI measurements with respect to the bleeding index. However, GI was recorded by the same, well-calibrated examiners in both first and repeat trials, with inter- and intra-examiner k values >0.50. Therefore, it can be speculated that differences in GI recordings as observed between trials may be ascribed to true, time-dependent differences in the clinical expression of gingival inflammatory response to similar plaque accumulation in the considered population.

The present study also found that significant differences in gingival inflammation after 21 days of experimental gingivitis between the two groups of subjects are consistent with similarly significant gingival inflammation differences between the groups when observed in their ''natural state'' (i.e., at day 14). This was true for the absolute GI values of the test quadrant (Table 5), as well as the whole-mouth GI/PlI ratio. These findings, which are consistent with the findings of Abbas et al. (1986) who used the bleeding/plaque ratio as indicator, suggest that it might be possible to select a priori, on the basis of presenting level of gingival inflammation, subjects with different degree of gingival inflammatory response to de novo plaque accumulation.

After the first trial, the identification of two subpopulations of individuals (LR and HR) presenting different gingivitis susceptibility was based on the standardization of the GCF volume on the CPE. After 21 days of plaque accumulation, HR individuals, compared with LR individuals, showed significantly higher values for all the clinical parameters of gingival inflammation despite the lack of any difference in PlI or CPE between the two groups (Trombelli et al. 2004c). In the present study, due to differences in the methodology followed for GCF sampling, it was not possible to use GCF volume as the primary outcome variable to determine the severity of gingival inflammation and, consequently, the individual variability in the inflammatory response to plaque. GI was also preferred for determining the severity of gingival inflammation as it is widely used in clinical practice, sensitive and highly correlated with the extent and rate of plaque accumulation (Tatakis & Trombelli 2004, Trombelli et al. 2004c). A derived parameter was used, i.e., the day 21 (log e GI)/(log e CPE) – day 21, to identify two subsets of individuals with low and high susceptibility to plaque-induced gingival inflammation (defined as LS and HS, respectively). Interestingly, after the first trial, the day 21 test quadrant log e GI/log e CPE, as observed in 17 LR and 20 HR subjects included in the repeat trial, was significantly different between the groups ($p = 0.002$, data not shown). Consistently, this difference was similar to the inter-group difference in day 21 test quadrant log e GI/log e CPE, as observed in the entire LR ($n = 24$) and HR ($n = 24$) groups ($p = 0.0002$, data not shown). Overall, these observations seem to suggest that both the methods, the standardized GCF/CPE and log e GI/log e CPE ratio, may be sensitive to discriminate subpopulations with different susceptibility to plaque-induced

gingival inflammation. However, it is not possible at this time to determine if, and to what extent, differences between these two methods may have adversely affected the ability to identify consistent responders between the two trials.

When the day 21 test quadrant log e GI/log e CPE distribution for the entire repeat trial population (17 HR and 20 LR) was used to identify subjects in the lower (LS) and upper (HS) half of the distribution, 20 subjects (54%) were classified in the same (low or high, respectively) group. These data echo the findings of a previous analysis, which indicated that 50% of LR and 71% of HR presented a consistent susceptibility to plaque-induced gingival inflammation at different time points during the course of the first trial (Trombelli et al. 2006a). The present results are also in agreement with the findings of van der Weijden et al. (1994), who reported that, among 25 dental students who participated in two experimental gingivitis trials performed 6 months apart, 16 subjects (64%) consistently exhibited either greater ($n = 10$) or less ($n = 6$) than average gingival inflammation, as assessed by a bleeding index. Overall, the results of these studies suggest that although a proportion of subjects are consistent in their response, additional studies of different design and methodology, and perhaps different indicators, will be needed to firmly establish what proportion of a population can be consistently characterized as HR or LR to plaque accumulation.

Acknowledgements

We are grateful to Drs. Marina Tosi, Elisa Orlandini and Sabrina Bottega, University of Ferrara, for their clinical service.

References

Abbas, F., van der Velden, U., Hart, A., Moorer, W. R., Vroom, T. M. & Scholte, G. (1986) Bleeding/plaque ratio and the development of gingival inflammation. *Journal of Clinical Periodontology* **13**, 774–782.

Bosman, C. W. & Powell, R. N. (1977) The reversal of localized experimental gingivitis. A comparison between mechanical toothbrushing procedures and a 0.2% chlorhexidine mouthrinse. *Journal of Clinical Periodontology* **4**, 161–172.

Daly, C. G. & Highfield, J. E. (1996) Effect of localized experimental gingivitis on early supragingival plaque accumulation. *Journal of Clinical Periodontology* **23**, 160–164.

Deinzer, R., Forster, P., Fuck, L., Herforth, A., Stiller-Winkler, R. & Idel, H. (1999) Increase of crevicular interleukin 1beta under academic stress at experimental gingivitis sites and at sites of perfect oral hygiene. *Journal of Clinical Periodontology* **26**, 1–8.

Lie, M. A., Danser, M. M., van der Weijden, G. A., Timmerman, M. F., de Graaff, J. & van der Velden, U. (1995) Oral microbiota in subjects with a weak or strong response in experimental gingivitis. *Journal of Clinical Periodontology* **22**, 642–647.

Löe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.

Löe, H., Theilade, E., Jensen, S. B. & Schiott, C. R. (1967) Experimental gingivitis in man. 3. Influence of antibiotics on gingival plaque development. *Journal of Periodontal Research* **2**, 282–289.

Matheny, J. L., Abrams, H., Johnson, D. T. & Roth, G. I. (1993) Microcirculatory dynamics in experimental human gingivitis. *Journal of Clinical Periodontology* **20**, 578–583.

Putt, M. S., van der Weijden, G. A., Kleber, C. J. & Saxton, C. A. (1993) Validation of a 21-day, partial-mouth gingivitis model for evaluating chemotherapeutic dentifrices. *Journal of Periodontal Research* **28**, 301–307.

Scapoli, C., Mamolini, E. & Trombelli, . L. (2007) Role of IL-6, TNF-A and LT-A variants in the modulation of the clinical expression of plaque-induced gingivitis. *Journal of Clinical Periodontology* **34**, 1031–1038.

Scapoli, C., Tatakis, D. N., Mamolini, E. & Trombelli, L. (2005) Modulation of clinical expression of plaque-induced gingivitis: interleukin-1 gene cluster polymorphisms. *Journal of Periodontology* **76**, 49–56.

Shearer, B., Hall, P., Clarke, P., Marshall, G. & Kinane, D. F. (2005) Reducing variability and choosing ideal subjects for experimental gingivitis studies. *Journal of Clinical Periodontology* **32**, 784–788.

Tatakis, D. N. & Trombelli, L. (2004) Modulation of clinical expression of plaque-induced gingivitis. I. Background review and rationale. *Journal of Clinical Periodontology* **31**, 229–238.

Theilade, E., Wright, W. H., Jensen, S. B. & Löe, H. (1966) Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *Journal of Periodontal Research* **1**, 1–13.

Trombelli, L., Farina, R., Manfrini, R. & Tatakis, D. N. (2004a) Modulation of clinical expression of plaque-induced gingivitis: effect of incisor crown form. *Journal of Dental Research* **83**, 728–731.

Trombelli, L., Scapoli, C., Calura, G. & Tatakis, D. N. (2006a) Time as a factor in the identification of subjects with different susceptibility to plaque-induced gingivitis. *Journal of Clinical Periodontology* **33**, 324–328.

Trombelli, L., Scapoli, C., Orlandini, E., Tosi, M., Bottega, S. & Tatakis, D. N. (2004b) Modulation of clinical expression of plaque-induced gingivitis. III. Response of ''high responders'' and ''low responders'' to therapy. *Journal of Clinical Periodontology* **31**, 253–259.

Trombelli, L., Tatakis, D. N., Scapoli, C., Bottega, S., Orlandini, E. & Tosi, M. (2004c) Modulation of clinical expression of plaque-induced gingivitis. II. Identification of ''high-responder'' and ''low-responder'' subjects. *Journal of Clinical Periodontology* **31**, 239–252.

van der Weijden, G. A., Timmerman, M. F., Danser, M. M., Nijboer, A., Saxton, C. A. & van der Velden, U. (1994) Effect of pre-experimental maintenance care duration on the development of gingivitis in a partial mouth experimental gingivitis model. *Journal of Periodontal Research* **29**, 168–173.

Watts, T. L. (1978) Variability of gingival bleeding in experimental gingivitis trials. *Community Dentistry and Oral Epidemiology* **6**, 253–255.

Address:
Prof. Leonardo Trombelli
Research Center for the Study of Periodontal Diseases
University of Ferrara
Corso Giovecca 203
44100 Ferrara
Italy
E-mail: l.trombelli@unife.it

Clinical Relevance

Scientific rationale for the study: Gingivitis susceptibility may vary significantly among subjects. At present, it is not clear whether or not gingivitis susceptibility is a reproducible trait.

Principal findings: Within the present model, the experimental gingivitis clinical parameters were shown to be, to some extent, reproducible. At least 50% of the study population demonstrated a consistent inflammatory response to experimentally induced de novo plaque accumulation.

Practical implications: The present findings could help to set the basis for an early identification of subjects highly susceptible to plaque-induced gingivitis.