



UNIVERSITÀ  
DEGLI STUDI  
FIRENZE

## FLORE

# Repository istituzionale dell'Università degli Studi di Firenze

### **RETROSPECTIVE EVALUATION OF THE INFLUENCE OF THE IL-1 GENOTYPE ON RADIOGRAPHIC BONE LEVELS IN TREATED**

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

*Original Citation:*

RETROSPECTIVE EVALUATION OF THE INFLUENCE OF THE IL-1 GENOTYPE ON RADIOGRAPHIC BONE LEVELS IN TREATED PERIODONTAL PATIENTS MAINTAINED OVER 10 YEARS / M. CATTABRIGA; R. ROTUNDO; L. MUZZI; M. NIERI; G. VERROCCHI; F. CAIRO; G. PINI PRATO. - In: JOURNAL OF PERIODONTOLOGY. - ISSN 0022-3492. - ELETTRONICO. - 72:(2001), pp. 767-773.

*Availability:*

The webpage <https://hdl.handle.net/2158/2712> of the repository was last updated on

*Terms of use:*

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

*Publisher copyright claim:*

La data sopra indicata si riferisce all'ultimo aggiornamento della scheda del Repository FloRe - The above-mentioned date refers to the last update of the record in the Institutional Repository FloRe

(Article begins on next page)

# Retrospective Evaluation of the Influence of the Interleukin-1 Genotype on Radiographic Bone Levels in Treated Periodontal Patients Over 10 Years

Marcello Cattabriga,\* Roberto Rotundo,† Leonardo Muzzi,† Michele Nieri,‡ Guerina Verrocchi,\* Francesco Cairo,† and Giovanpaolo Pini Prato†

**Background:** A difference in genetic susceptibility to plaque accumulation has been advocated to explain different responses to periodontal therapy. The purpose of this study is to assess the role of the interleukin-1 (IL-1) polymorphism on the rate of bone and tooth loss in non-smoking periodontally treated patients during maintenance.

**Methods:** Sixty consecutive non-smoking patients (mean age  $46.8 \pm 5.0$ ) with moderate to severe periodontitis, treated and maintained for over 10 years were selected. At baseline (T0), radiographic evaluation (cementoenamel junction [CEJ]-root apex, CEJ-bottom of defect mesial and distal, CEJ-bone crest mesial and distal, crown-root ratio) was performed. All patients received scaling and root planing; 36 patients then underwent surgical therapy. Subsequently, all patients were enrolled in a periodontal maintenance program with recall visits every  $3.4 \pm 1.0$  months for at least 10 years. At the latest recall visit (T2) the same radiographic measurements evaluated at baseline were taken and a DNA sample for IL-1 genetic susceptibility testing was collected and sent for analysis.

**Results:** Twenty-three of the 60 patients (38.3%) were IL-1 genotype positive. A total of 52 teeth (3.3%) out of 1,566 were lost due to periodontitis between T0 and T2; 28 of 957 (2.9%) in the IL-1 genotype negative group and 24 of 609 (3.9%) in IL-1 genotype positive group. The mean variation in bone defect level ( $\Delta$ BD) averaged  $-0.04$  mm in IL-1 genotype negative patients and  $0.01$  mm in IL-1 genotype positive patients. The mean variation in bone crest level ( $\Delta$ BC) averaged  $-0.24$  mm in IL-1 genotype negative patients and  $-0.28$  mm in IL-1 genotype positive patients. However, a few patients showed significant differences in response to therapy based on initial bone levels and genotype. IL-1 negative patients who showed minimal initial bone loss responded to the therapy better than the IL-1 positive patients. IL-1 positive patients with severe initial bone loss showed a better response to the therapy than IL-1 negative patients.

**Conclusions:** On average, there were no significant differences related to IL-1 genotype in tooth loss after 10 years in a non-smoking, well-maintained periodontal population. On an individual patient basis, the IL-1 genotype, in combination with the initial bone level, seems useful at the beginning of therapy for predicting bone level variation. *J Periodontol* 2001;72:767-773.

## KEY WORDS

Periodontal diseases/therapy; interleukin-1; bone loss/etiology; tooth loss/etiology; disease susceptibility.

Many studies have documented that periodontitis can be successfully treated provided good plaque control is achieved. However, not all sites or patients respond similarly to therapy. In long-term studies, a small percentage of patients have continued to break down leading to increased tooth loss.<sup>1</sup>

Different responses to plaque accumulation based on differences in genetic susceptibility have been advocated to explain this variation.

Recently a genetic test<sup>§</sup> has been developed to identify a specific genotype in the IL-1 gene cluster. Patients testing genotype positive produce increased levels of interleukin-1 in response to an inflammatory stimulus. The increased production of this inflammatory mediator can lead to increased destruction of periodontal tissues following plaque accumulation. It has been calculated that 29.1% of Caucasian Northern European subjects are IL-1 genotype

\* Private practice, Rome, Italy.

† Dental School, University of Florence, Italy.

‡ Private practice, Florence, Italy.

§ PST, Interleukin Genetics, Waltham, MA.

positive and, for this reason, this group of patients should be considered at major risk to develop severe periodontal disease.<sup>2</sup>

In the Kornman et al. cross-sectional study,<sup>2</sup> the IL-1 genotype in non-smoking patients between the ages of 40 and 60 was strongly related to severe periodontitis with an odds ratio of 18.90 (severe versus mild periodontitis). In smokers, however, severe disease was not correlated with the IL-1 genotype, confirming the importance of smoking alone as an important risk factor.<sup>2</sup>

A recent longitudinal retrospective evaluation by McGuire and Nunn<sup>3</sup> has confirmed the important role of smoking and a positive IL-1 genotype in relation to tooth loss in a group of patients who have been in maintenance care for 14 years. The authors showed that a positive IL-1 genotype increased the risk of tooth loss by 2.7 times, heavy smoking by 2.9 times, and the combined effect of positive IL-1 genotype and heavy smoking by 7.7 times.

These studies indicate that not only is there an association between IL-1 genotype positive patients and severe periodontal disease, but also that tooth mortality rate is higher in this group of patients even after treatment and appropriate maintenance.

The objective of this investigation is to evaluate if the IL-1 genotype impacts radiographic bone levels and tooth loss in non-smoking patients, aged 40 to 60, following treatment and long-term periodontal maintenance.

## MATERIALS AND METHODS

The study population consisted of 60 patients consecutively selected by a single clinician in a single private periodontal practice during a routine periodontal maintenance visit (PMV). Patients had to satisfy the following criteria: 1) moderate to severe periodontitis prior to treatment with the presence of at least 15 teeth, excluding third molars; 2) previous periodontal treatment; 3) 40 to 60 years old at the beginning of therapy; 4) non-smoker for at least 5 years prior to treatment and throughout subsequent years of maintenance; 5) negative medical history; and 6) in periodontal maintenance for at least 10 years.

Patients included 29 males (48%) and 31 females (52%), of Caucasian heritage, from the middle economic levels and a mean age of  $46.8 \pm 5.0$  years (range 40 to 58) at the beginning of treatment. All subjects were treated and maintained for at least 10 years ( $12.7 \pm 2.2$  years, mean  $\pm$  SD) between 1980 and 1999.

### Treatment

The same operator, with more than 10 years of experience, treated patients in the same private practice limited to periodontics.

At the baseline (T0), all patients were clinically and radiographically evaluated and thoroughly instructed in proper oral hygiene methods (modified Bass technique and interproximal cleaning procedures). Subjects were treated with full-mouth scaling and root planning under local anesthesia in 4 appointments. Five to 7 weeks after initial treatment, patients were clinically re-evaluated (T1).

Based on clinical judgment and the nature of the periodontal defect, 36 subjects were subsequently treated surgically with modified Widman flap or resective surgery. During the PMV phase, subgingival instrumentation was performed under local anesthesia at specific sites if bleeding on probing was evident at a recall appointment. If bleeding on probing was still present at the following recall visit or there was a negative change in probing depth or x-rays evaluation, surgery was scheduled.

All subjects participated in a stringent PMV program. At each recall appointment (mean  $\pm$  SD,  $3.4 \pm 1.0$  months), they received a full-mouth professional prophylaxis and localized subgingival instrumentation as needed. Oral hygiene procedures (including interproximal cleaning) and patient compliance were reinforced each visit. Probing depths were recorded every year and an x-ray examination performed every 3 years.

After 10 years minimum of PMV (T2), the subjects were clinically and radiographically re-evaluated. During this maintenance period, any additional prosthetic, endodontic, and restorative treatments or tooth extractions were performed by different operators in consultation with the periodontist.

### Clinical Evaluation

At the T0 and T2, a dental examination recording the number of missing teeth, restorations, and x-ray status was performed. No third molars were included in this study. In addition, at the T2 appointment, a fingerstick blood sample was collected and submitted for IL-1 genotype analysis. Each subject's finger was cleaned with an antiseptic wipe and the skin was punctured with a sterile lancet to collect blood droplets on a DNAase-free blotting paper and sent for blinded analysis to the genetic testing laboratory. The results of the test (genotype positive or negative) were not revealed until all the clinical and radiographic evaluations were complete (T2).

### Radiographic Evaluation

For the purpose of this study, T0 and T2 intra-oral radiographs<sup>||</sup> were analyzed. The radiographs were obtained with a film holder<sup>¶</sup> using the long-cone technique. Radiographs were placed on a diaphanoscope

<sup>||</sup> Ultra-speed DF 58, Eastman Kodak Company, Rochester, NY.

<sup>¶</sup> Rinn XCP, Rinn Corporation, Elgin, IL.

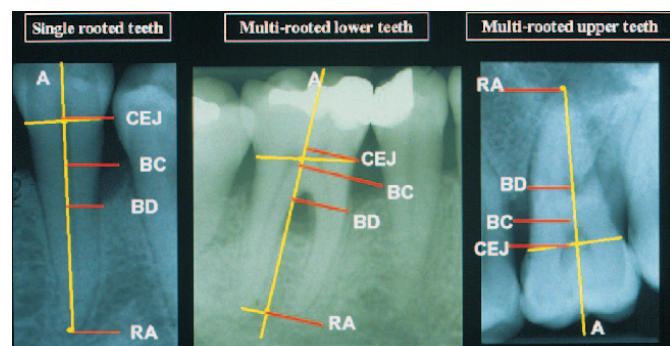
without any magnification and a millimeter ruler used directly on the films to obtain the linear measurements (to the next mm). Two operators evaluated all radiographic measurements. The measurement criteria were based on standardized reference points and on a tooth referring axis (Fig. 1).

The referral points were the cemento-enamel junction (CEJ), both mesial and distal, and the root apex (RA). Mesial and distal measurements for lower molars were obtained by using the median point of the conjunction line of the 2 apices of the mesial and distal roots. For the upper molars, the apex of palatal root for both the mesial and distal measurements was used. For upper and lower single rooted teeth, the unique apex was used. The bone defect (BD) was the deepest point of the defect, mesial and distal and the bone crest (BC), the most coronal point, mesial and distal.

On single-rooted teeth, the referral axis was obtained by connecting the root apex point with the median point of CEJmes-CEJdist line. On lower molars, the axis was obtained by connecting the median point of the apex line with the median point of CEJmes-CEJdist line. On upper molars, the axis was obtained connecting the apex point of palatal root with the median point of CEJmes-CEJdist line.

The following measurements were taken by projecting these referral points on the main axis and measuring the following distances: 1) CEJ-RA (mesial and distal); 2) CEJ-BD (mesial and distal); and 3) CEJ-BC (mesial and distal).

If the reference points were not detectable on the x-ray film, due to the overlapping of images or points out of film view, the respective measurements at the missing point were excluded. When the CEJ point was not detectable because of a restoration (filling or prosthetic crown), the margin of that restoration was used as the reference point.



**Figure 1.**

Radiographic evaluation technique. The measurements were taken reporting the projection of the referral points on the main axis.

**A:** referral axis; **CEJ:** cemento-enamel junction; **BC:** bone crest; **BD:** bone defect; **RA:** root apex.

### Statistical Analysis

The reliability of the radiographic measurements was evaluated with a preliminary intra- and inter-examiner reliability study by evaluating the same measurement of the same radiograph at 2 different times. Intra-class correlation coefficients (R), considering raters fixed, were determined for: 1) the reproducibility of measurements made by each observer; and 2) the measurements made by one observer versus the other. The measurements evaluated were CEJ-RA mesial and distal, CEJ-BD mesial and distal, and CEJ-BC mesial and distal. The number of analyzed random sites was approximately 50 for each variable.<sup>4,5</sup> An important inferential question was whether the rater effects differ significantly from one another.

Descriptive analysis was performed on the independent variable data for all the available sites averaged for each patient. Means at baseline were evaluated for both IL-1 genotype negative and positive patients and compared using the *t*-test, Wilcoxon test, or Fisher exact test according to the variable analyzed.

The measurements recorded at T2 were related to the root length in order to correct, as much as possible, errors due to projection of the mesial as to distal sites. The following equations were used:

$$\Delta BD = CEJ-BD_{T0} - CEJ-BD_{T2} \times \frac{CEJ-RA_{T0}}{CEJ-RA_{T2}}$$

$$\Delta BC = CEJ-BC_{T0} - CEJ-BC_{T2} \times \frac{CEJ-RA_{T0}}{CEJ-RA_{T2}}$$

Two statistical analyses were performed on the means for  $\Delta BD$  and  $\Delta BC$  for the IL-1 genotype negative and positive patients to evaluate differences in therapy effectiveness by genotype.

Teeth lost during the study, teeth with mesial or distal CEJ-RA differences  $\geq 5$  mm between T0 and T2, teeth restored during the maintenance phase, teeth with renewed restorations, and teeth for which it was impossible to measure CEJ-RA or CEJ-BD were excluded from the statistical analysis performed on  $\Delta BD$ . Similar exclusion criteria were considered for  $\Delta BC$  analysis. Patients with at least 20 measurable sites were included in the analysis. The remaining sites were analyzed for IL-1 genotype negative and positive groups. All sites for the patient were averaged so that the analysis was conducted at the patient level.

A *t*-test was used to compare the 2 genotype groups at baseline. An analysis of covariance (ANCOVA) was used on the  $\Delta BD$  and  $\Delta BC$  values, correcting for the baseline BD and BC values, respectively. The ANCOVA model included a test for heterogeneity of slopes (a test of the hypothesis of parallel regression lines). If the lines were parallel, then a test for group differences would have been performed. Since the lines were not parallel, the Potthoff extension of the Johnson-

Neyman procedure<sup>6-10</sup> was used. This procedure provides regions of significance where the groups are different, depending on the baseline covariate.

## RESULTS

Preliminary study results concerning variables mesial CEJ-RA, distal CEJ-RA, mesial CEJ-BD, distal CEJ-BD, mesial CEJ-BC, and distal CEJ-BC did not show a significant interexaminer reliability difference ( $\alpha = 0.05$ ) and the intra-class correlation coefficients (R) were all  $>0.85$  (Table 1).

Results of the genetic test indicated 23 patients were IL-1 genotype positive (38.3%) and 37 negative (61.7%). Descriptive statistical analysis data are shown in Table 2.

**Table 1.**

### Results of Preliminary Intra- and Interexaminer Reliability Study

	CEJ-RAmes	CEJ-RAdis	CEJ-BDmes	CEJ-BDdis	CEJ-BCmes	CEJ-BCdis
Examiner 1	0.98	0.97	0.99	0.99	0.99	0.98
Examiner 2	0.86	0.90	0.95	0.95	0.96	0.89
Interexaminer	0.90	0.94	0.97	0.92	0.96	0.92

**Table 2.**

### Descriptive Statistical Analysis (data averaged on all valuable sites; mean $\pm$ SD)

	IL-1 Negative N = 37	IL-1 Positive N = 23	P
Age	46.03 $\pm$ 4.21 (40-53)*	47.96 $\pm$ 5.88 (40-58)	0.1442 (t test)
N = females	18	13	0.6033 (Fisher exact test)
T0 teeth	25.86 $\pm$ 2.49 (17-28)	26.48 $\pm$ 1.88 (21-28)	0.4667 (Wilcoxon test)
Tooth loss (due to periodontitis)	0.76 $\pm$ 0.93 (0-3)	1.04 $\pm$ 1.15 (0-4)	0.3565 (Wilcoxon test)
T0 restored teeth	2.19 $\pm$ 2.93 (0-11)	1.43 $\pm$ 2.04 (0-7)	0.2846 (t test)
Surgically treated teeth	7.00 $\pm$ 8.79 (0-28)	7.96 $\pm$ 9.58 (0-26)	0.6936 (t test)
CEJ-RA <sub>T0</sub> (mm)	15.71 $\pm$ 1.50 (13.27-18.76)	15.96 $\pm$ 1.32 (13.96-18.90)	0.5144 (t test)
CEJ-BD <sub>T0</sub> (mm)	4.17 $\pm$ 1.21 (1.37-6.76)	4.23 $\pm$ 1.23 (2.38-6.43)	0.8454 (t test)
CEJ-BC <sub>T0</sub> (mm)	3.37 $\pm$ 1.05 (1.17-6.09)	3.22 $\pm$ 0.99 (1.71-5.11)	0.5842 (t test)

\* Minimum to maximum values.

As for tooth loss, 52 teeth (3.3%) out of 1,566 were lost due to periodontitis between baseline and T2: IL-1 genotype negative patients lost 28 teeth (2.9%) out of 957 present at T0 (an average of 0.76 teeth for patient) and IL-1 positive patients lost 24 teeth (3.9%) out of 609 present at T0 (an average of 1.04 teeth for patient). These differences were not statistically significant. Over half of the patients (36/60) had subsequent surgical procedures performed during the maintenance period. Of these, 22 were from the genotype negative group (22/37 or 59.5%) and 14 from the genotype positive group (14/23 or 60.9%). The average number of surgically treated teeth per patient was 7.00 for genotype negative patients and 7.96 for genotype positive patients. The difference was not statistically significant ( $P = 0.6936$ ).

At the beginning of therapy 1,566 teeth (3,132 sites) were originally present. Subsequently 189 teeth were excluded. The remaining 1,377 teeth (2,754 sites) were included in the analysis (Table 3). Two IL-1 negative patients were excluded because they had less than 20 sites radiographically detectable.

From the  $\Delta$ BD calculation, 362 sites were excluded and the remaining 2,392 sites were then averaged for patient. For the  $\Delta$ BC calculation, 365 sites were excluded and the remaining 2,389 sites were then averaged for patient. The average number of sites per patient used for  $\Delta$ BD and  $\Delta$ BC analysis is shown in Table 4.

The results for  $\Delta$ BD (averaged for patient) in the IL-1 genotype negative group were  $-0.04$  mm  $\pm$  0.46 and  $0.01$  mm  $\pm$  0.61 for the IL-1 genotype positive group. The results for  $\Delta$ BC (averaged for patient) were  $-0.24$  mm  $\pm$  0.49 for the IL-1 genotype negative group and  $-0.28$  mm  $\pm$  0.36 for the IL-1 genotype positive group.

The 2 tests of hypothesis of parallel regression lines are statistically significant (IL-1 genotype  $\times$  CEJ-BD<sub>T0</sub> interaction:  $P$  value = 0.0051; IL-1 genotype  $\times$  CEJ-BC<sub>T0</sub> interaction:  $P$  value = 0.0054). Since the slopes of regression were unparallel, Potthoff's technique has been applied. The lower boundary (XL) and upper boundary (XU) of the region of non-significance were identified (Table 5). A scatter plot with the regression lines of the 2 genotype groups for  $\Delta$ BD and  $\Delta$ BC in relation to the baseline CEJ-BD and CEJ-BC values is shown in

**Table 3.**  
**Teeth and Sites Excluded From the Study**

	Teeth (1,566)	ΔBD Sites (3,132)	ΔBC Sites (3,132)
Teeth lost to periodontitis	52 (3.32%)*	104 (3.32%)	104 (3.32%)
Restored teeth	127 (8.11%)	254 (8.11%)	254 (8.11%)
CEJ-RA <sub>T0</sub> -CEJ-RA <sub>T2</sub>   ≥5 mm	10 (0.64%)	20 (0.64%)	20 (0.64%)
Sites not detectable on radiographs (CEJ <sub>T0-2</sub> , RA <sub>T0-2</sub> , BD <sub>T0-2</sub> , BC <sub>T0-2</sub> )		348 (11.11%)	351 (11.21%)
N sites in 2 patients excluded		14 (0.45%)	14 (0.45%)
Remaining sites		2,392 (76.37%)	2,389 (76.28%)

\* Based on number of teeth and sites at baseline.

**Table 4.**  
**Average Number of Sites Per Patient Used for ΔBD and ΔBC Analysis (mean ± SD)**

	IL-1 Negative N = 35	IL-1 Positive N = 23	P (t test)
N sites for ΔBD analysis	41.77 ± 7.79 (25-54)*	40.43 ± 8.04 (21-54)	0.5306
N sites for ΔBC analysis	41.71 ± 7.80 (25-54)	40.39 ± 8.06 (21-54)	0.5353

\* Minimum to maximum values.

**Table 5.**  
**Covariate Values of the Lower (XL) and Upper Boundary (XU) of the Region of Non-Significance in Relation to the Baseline CEJ-BD and CEJ-BC Values**

	XL	XU
CEJ-BD <sub>T0</sub> (mm)	1.70	5.81
CEJ-BC <sub>T0</sub> (mm)	2.00	5.52

Figures 2 and 3, respectively. The covariate values within the range XL-XU were not statistically significant between positive and negative IL-1 genotype patients.

However, as evident from the ΔBD scatter plot (Fig. 2), of the 23 patients who were IL-1 genotype positive, there were only 3 above the 5.81 cut-off value. Of the 35 patients who were IL-1 genotype negative, there was 1 patient above the upper boundary and 1 patient below the lower boundary of the region of non-significance. As for the ΔBC scatter plot (Fig. 3), 2 IL-1 genotype positive patients were below the lower boundary limit. For the IL-1 genotype negative patients, 2

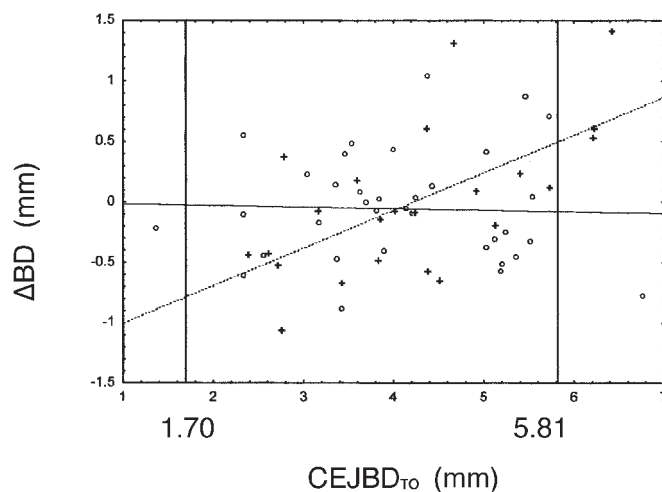
were below the lower boundary and 1 was above the upper boundary of the region of non-significance.

**DISCUSSION**

The aim of the present study was to evaluate the effect of positive versus negative genotype patients (IL-1 polymorphism) on bone level variations and on tooth loss following periodontal therapy and long-term maintenance. For these purposes, 60 consecutively selected periodontal patients, treated and maintained on a 3-month recall program for more than 10 years, were examined.

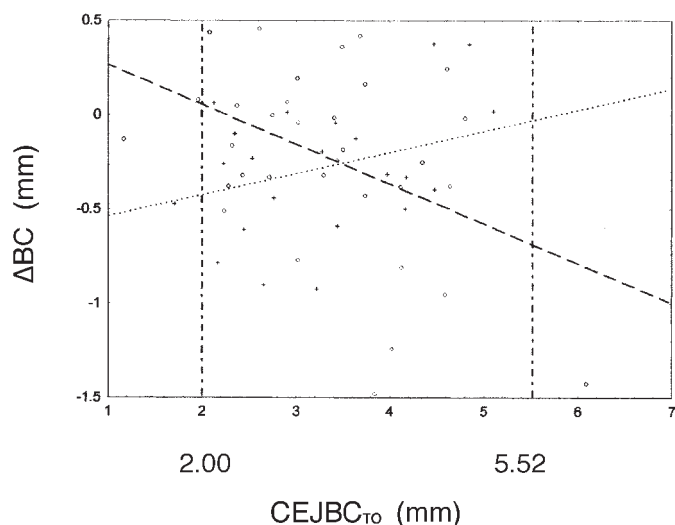
All 60 patients had the IL-1 genetic test in which 23 (38.3%) were genotype positive. These prevalence data are in accordance with those of other studies.<sup>2,3,11-13</sup>

A recent study by McGuire and Nunn<sup>3</sup> reported on the influence of IL-1 polymorphism genotype within a population treated and maintained long term. Their data showed 47 teeth lost for periodontal reasons (4.6%) in 42 patients, of which 27 (7%) were lost in IL-1 genotype positive patients and 20 teeth (3%) in IL-1 genotype negative patients. Unfortunately, given



(IL-1 genotype negative)  $\Delta BD = -0.02 - 0.01 \text{ CEJ-BD}_{T0}$   
 (IL-1 genotype positive)  $\Delta BD = -1.30 + 0.31 \text{ CEJ-BD}_{T0}$

**Figure 2.**  
Scatter plot and regression lines for the variation of the bone defect level (ΔBD) for IL-1 genotype positive (+) and negative patients (o).  
 — = regression line IL-1 genotype negative.  
 ..... = regression line IL-1 genotype positive.



(IL-1 genotype negative)  $\Delta BC = 0.46 - 0.21 \text{ CEJBC}_{T0}$

(IL-1 genotype positive)  $\Delta BC = -0.64 + 0.11 \text{ CEJBC}_{T0}$

### Figure 3.

Scatter plot and regression lines for the variation of the bone crest level ( $\Delta BC$ ) for IL-1 genotype positive (+) and negative patients (○).

----- = regression line IL-1 genotype negative.

..... = regression line IL-1 genotype positive.

the differences in study design and population characteristics (30 patients in the McGuire and Nunn study<sup>3</sup> were or had been smokers), it was impossible to directly compare the results.

In the present investigation of 60 subjects, the percentage tooth loss overall was 3.3% (52 teeth); of which 24 teeth were lost in IL-1 genotype positive group and 28 teeth in IL-1 genotype negative group. The mean tooth loss was not significantly different between the 2 groups. Furthermore, the difference in the average number of teeth requiring surgical intervention per patient was not statistically significant by genotype. Of the 36 patients who underwent surgery, 22 were genotype negative patients (59.5%) and 14 were genotype positive patients (60.9%). The mean number of teeth surgically treated per patient was 7.00 and 7.96 for IL-1 genotype negative and positive patients, respectively. This difference was not statistically significant. It should also be noted that surgery was performed after initial therapy or sometime during the maintenance period. The philosophy of the periodontal practice was to perform surgery during maintenance when one or more sites were not stable in terms of probing depth or x-ray evaluation. Moreover, if bleeding on probing was evident at a recall appointment, subgingival instrumentation was performed under local anesthesia at those sites. If bleed-

ing on probing was still present at the following SPC appointments, especially in previously unstable sites, surgery was performed.

The data from our study referring to the initial bone levels in positive and negative IL-1 genotype patients indicate that the 2 groups of patients were initially homogeneous since no statistical significance could be demonstrated. This was surprising because one would expect more destruction in the IL-1 positive group. This could be related to the initial entry criteria that probably rendered the 2 groups equivalent at the beginning of the study.

As for the bone level variations radiographically assessed at both T0 and T2, the results could be affected by the measuring technique. However, the preliminary reliability test was performed to assess the intra- and inter-examiner reliability and it was acceptable. In addition, the measurements were related to the root length value in order to reduce the error due to different radiographic projection and changes over time such as root resorption. Some sites were excluded because the reference points were not always detectable due to new restorations (254); the inability to locate radiographically the exact position of the CEJ, root apex or bone defect (348); tooth extractions (104); or differences between T0 and T2 measurements where the  $|CEJ-RA_{T0} - CEJ-RA_{T2}| \geq 5 \text{ mm}$  (20). For the latter exclusion criterion, a threshold value of 5 mm was chosen to avoid errors related to marked root resorptions or different radiographic projections for which the correction calculation would not report satisfactory results. Our corrected absolute change allowed us to use measurements in millimeters instead of in percentage.

The Potthoff's analysis revealed some unclear effects of IL-1 polymorphism regarding the variation of the bone levels in relation to the data at baseline, in the few patients who were outside the region of non-significance. In particular, IL-1 genotype negative patients who showed minimal initial bone loss responded to the therapy better than the IL-1 positive patients. However, IL-1 genotype positive patients with a severe initial bone loss showed a better response to the therapy than IL-1 genotype negative patients. With this finding, the genetic susceptibility testing in conjunction with the information of initial bone loss profile (slight or severe) seems to be particularly useful for prognostic reasons at the beginning of therapy for predicting treatment response.

Our population is obviously a group of patients who are highly committed, compliant, and satisfied with their therapy; however, the aim of our study was to consider the differences between the IL-1 genotype positive and negative patients who followed a meticulous maintenance program. In addition, the genetic evaluation was performed after all the dental measures

were recorded and the clinician was blinded to the genetic results. The fact that the slope of the regression lines was different seems to indicate that periodontal therapy is effectively different in the 2 groups, especially for the extreme values of initial bone loss, out of the region of non-significance. For intermediate values, the therapy seems equally effective in the 2 groups.

However, in a study by De Sanctis and Zucchelli<sup>13</sup> the authors evaluated the influence of the interleukin-1 gene polymorphism on the long-term stability of patients (8 smokers, 32 non-smokers) treated by guided tissue regeneration (GTR). The results demonstrated that genotype positive expression did not affect the outcome of GTR treatment at 1 year, but had a greater impact on long-term stability at 4 years. These results seem to support the findings of another recent study by Socransky et al.<sup>14</sup> where the authors showed that IL-1 genotype positive subjects more frequently have higher levels of harmful species of bacteria that are known to be strongly associated with periodontal inflammation than IL-1 genotype negative subjects. For this reason, the authors stated that strong management of the modifying factors with a more aggressive therapy and a stringent maintenance protocol of plaque control seem to be indicated in these high risk individuals.

In conclusion, on average, there were no significant differences by IL-1 genotype in tooth loss after 10 years in a non-smoking, well-maintained periodontal population. On an individual patient basis, the IL-1 genotype, in combination with the initial bone level, seems useful at the beginning of therapy for predicting bone level variations.

## ACKNOWLEDGMENT

The authors would like to acknowledge Nancy Whitley for her help in reviewing the English language of the manuscript.

## REFERENCES

1. Hirschfeld L, Wasserman B. A long term survey of tooth loss in 600 treated periodontal patients. *J Periodontol* 1978;49:225-237.
2. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;24:72-77.
3. McGuire MK, Nunn ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and interleukin-1 genotype in accurately predicting prognoses and tooth survival. *J Periodontol* 1999;70:49-56.
4. Fleiss JL. *The Design and Analysis of Clinical Experiments*. New York: John Wiley & Sons; 1986:1-26.
5. Dunn G. *Design and Analysis of Reliability Studies. The Statistical Evaluation of Measurement Error*. New York: Oxford University Press; 1989:34-35.
6. Potthoff RF. On the Johnson-Neyman technique and some extension thereof. *Psychometrika* 1964;29:241-256.
7. Johnson DR, Neyman J. Tests of certain linear hypotheses and their applications to some educational problems. *Stat Res Memoirs* 1936;1:57-93.
8. Huitema BE. *The Analysis of Covariance and Alternatives*. New York: Wiley-Interscience Publication; 1980: 270-297.
9. Pedhazur EJ. *Multiple Regression in Behavioral Research: Explanation and Prediction*, 3<sup>rd</sup> ed. Fort Worth: Harcourt Brace College Publishers; 1997:591-603.
10. Rogosa D. Comparing nonparallel regression lines. *Psycholog Bull* 1980;88:307-321.
11. Wilson TG Jr., Nunn ME. The relationship between the interleukin-1 periodontal genotype and implant loss. Initial data. *J Periodontol* 1999;70:724-729.
12. McDevitt MJ, Wang HY, Knobelmann C, et al. Interleukin-1 genetic association with periodontitis in clinical practice. *J Periodontol* 2000;71:156-163.
13. De Sanctis M, Zucchelli G. Interleukin-1 gene polymorphisms and long-term stability following guided tissue regeneration therapy. *J Periodontol* 2000;71:606-613.
14. Socransky SS, Haffajee AD, Smith C. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. *J Dent Res* 1999;78(Spec. Issue):872(Abstr. 3600).

Send reprint requests to: Dr. Giovanpaolo Pini Prato, 11 Viale Matteotti, 50121 Florence, Italy. Fax: 3955572881; e-mail: gpini@tin.it

Accepted for publication January 4, 2001.