J Clin Periodontol 2011; 38: 428–433 doi: 10.1111/j.1600-051X.2011.01701.x

Journal of Clinical Periodontology

Effect of periodontal treatment on receptor activator of NF-κB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid

Nagihan Bostanci[1], Buket Saygan[2], Gülnur Emingil[2], Gül Atilla[2] and Georgios N. Belibasakis[1]

[1]Center of Dental Medicine, Institute of Oral Biology, Faculty of Medicine, University of Zürich, Zürich, Switzerland; [2]Department of Periodontology, School of Dentistry, Ege University, İzmir, Turkey

Bostanci N, Saygan B, Emingil G, Atilla G, Belibasakis GN. Effect of periodontal treatment on receptor activator of NF-κB ligand and osteoprotegerin levels and ratio in gingival crevicular fluid. J Clin Periodontol 2011; 38: 428–433. doi: 10.1111/j.1600-051X.2011.01701.x.

Abstract

Aim: Receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) have an established role in the pathogenesis of periodontitis, which is characterized by an increased RANKL/OPG ratio. The present study aims to investigate changes of RANKL, OPG and their relative ratio in gingival crevicular fluid (GCF) of periodontitis patients after non-surgical periodontal treatment.

Materials and Methods: GCF was obtained from chronic periodontitis ($n = 14$), generalized aggressive periodontitis (G-AgP; $n = 13$) patients at baseline. The patients received scaling and root planing and were recalled after 2, 3 and 4 months for follow-up clinical examination and sampling. The total amounts and concentrations of RANKL and OPG in GCF were measured by enzyme-linked immunosorbent assay, and their relative ratio was calculated.

Results: The RANKL/OPG ratio remained unchanged and did not correlate with clinical parameters throughout the monitoring period, despite the improved clinical outcome. This trend was similar in both chronic and G-AgP.

Conclusions: Although the RANKL/OPG ratio has a potential diagnostic value for untreated periodontitis, it may not be a suitable predictor of clinically successful treatment outcome. As conventional therapy does not negatively modulate this ratio, the host could still be susceptible to further periodontal tissue destruction, warranting the consideration of adjunctive treatments.

Key words: ELISA; gingival crevicular fluid; OPG; periodontal treatment; RANKL; RANKL/OPG ratio

Accepted for publication 3 January 2011

Alveolar bone loss is a key clinical feature of periodontitis. On the molecular level, bone resorption is regulated by the interplay of receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG), two molecules belonging to the tumour necrosis factor ligand and receptor families, respectively (Teitelbaum & Ross 2003). On the cellular level, RANKL is expressed predominantly as a membrane-bound or secreted ligand by osteoblasts, fibroblasts and activated T and B cells (Liu et al. 2010). By activating its cognate RANK receptor on osteoclast precursors (cells of the monocyte/macrophage lineage), it triggers their fusion and differentiation into multi-nucleated osteoclasts, which will resorb bone (Lacey et al. 1998). On the contrary, OPG is a soluble inhibitor that binds to RANKL, thus preventing osteoclast differentiation and bone resorption (Simonet et al. 1997). RANKL and OPG production is regulated by several systemic and local stimuli, including hormones, inflammatory mediators and bacterial products (Lerner 2006).

Conflict of interests and source of funding statement

The authors state that they have no conflict of interest. This study was in part supported by the IADR/Philips Oral Healthcare Young Investigator Research Grant of the Periodontal Research Group (N. B.).

There is now a wide body of convincing evidence for the role of the RANKL–OPG system in human periodontitis (Taubman et al. 2007), that could potentially be exploited for diagnostic or therapeutic purposes (Bartold et al. 2010). Recent clinical studies have confirmed that both RANKL and OPG can be detected in human gingival crevicular fluid (GCF). These studies collectively indicate that RANKL levels are increased, whereas OPG levels are decreased in GCF from periodontitis-affected sites (Mogi et al. 2004, Vernal et al. 2004, Lu et al. 2006, Bostanci et al. 2007, Tang et al. 2009). An increase in the relative RANKL/OPG ratio is considered indicative of the occurrence of periodontitis (Bostanci et al. 2007). These cross-sectional studies have validated the RANKL–OPG system as a potential molecular diagnostic marker for periodontitis. However, there is at present only little information on the potential quantitative changes in this system in response to periodontal therapy. Therefore, the aim of this study was to investigate the effect of non-surgical periodontal treatment on RANKL, OPG and RANKL/OPG ratio levels in GCF of patients with periodontitis, over a period of 4 months.

Materials and Methods

Study population and clinical examination

A total of 27 patients were included in this study. Pre-screening examination was conducted to evaluate patient eligibility for inclusion in the study. Patients eligible for the study returned to the clinic at screening visit for clinical measurements, 1 week after pre-screening. All subjects were recruited from the Department of Periodontology (School of Dentistry, Ege University, İzmir, Turkey). The study was approved by the Ethics Committee of the Medical Faculty of Ege University and was conducted according to the guidelines of the World Medical Association Declaration of Helsinki. Written and informed consent was obtained from each patient before enrolment in the study. Complete medical and dental histories were taken from all patients. Systemic exclusion criteria were smoking, cardiovascular and respiratory diseases, diabetes mellitus, hepatitis or HIV infection, immunosuppressive chemotherapy and current pregnancy or lactation. None of the patients had taken medication such as antibiotics or contraceptives that could affect their periodontal status for at least 3 months before the study. The selection of the patients was made according to the clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage 1999). The periodontal status of each patient was assessed by a single calibrated examiner having experience in clinical trials (B. S.). The intra-examiner reproducibility for probing pocket depth (PPD) measurements was assessed and the inter-class correlation coefficient was 0.985 (95% confidence interval: 0.968–0.993). To determine the clinical periodontal status, all subjects had a clinical periodontal examination including the measurement of PPD and clinical attachment level (CAL) at six sites around each tooth with a manual probe (Williams probe). The full-mouth papilla bleeding index (PBI) and plaque index (PI) were also recorded. The patients were classified in two groups based on diagnosis: (a) a generalized aggressive periodontitis (G-AgP) group ($n = 13$, eight females and five males) with mean age 28.8 ± 5.7 years (between 18 and 36 years), demonstrating a generalized pattern of severe periodontal destruction and CAL $\geqslant$ 4 mm on eight or more teeth; at least three of those were other than central incisors or first molars, which was not consistent with patients' age, amount of plaque accumulation or local contributing factors, and (b) a chronic periodontitis (CP) group ($n = 14$, six females and eight males) with mean age 44.7 ± 5.0 years (between 35 and 52 years), exhibiting at least four sites with a PPD $\geqslant$ 6 mm and CAL $\geqslant$ 4 mm at the same site.

Periodontal treatment protocol

After the pre-screening and screening visits, the patients received non-surgical therapy starting at baseline with full-mouth supragingival scaling, followed by four weekly sessions of quadrant root planing under local anaesthesia up to 1 h until they were free of all deposits as determined by visual or tactile examination. Oral hygiene instructions including brushing, flossing and interdental brushing were given at baseline and reinforced at each recall session. The recall visits occurred at 2, 3 and 4 months after completion of the treatment. These were performed by the same examiner and consisted of GCF sampling, clinical measurements and reinforcement of oral hygiene procedures.

Collection of GCF

At baseline and each follow-up timepoint, GCF samples were collected from the mesiobuccal aspect of a single-rooted tooth exhibiting PPD of 5–8 mm. The selected sites were cleared of supragingival plaque, isolated with cotton rolls and dried with a gentle stream of air to prevent saliva contamination. A sterile Periopaper strip (ProFlow Inc. Amityville, NY, USA) was gently inserted into the periodontal pocket until mild resistance was felt and left in place for 30 s. Mechanical irritation was avoided and strips contaminated with blood were discarded.

The GCF sample volume was measured with a calibrated Periotron 8000 (Proflow Inc.) and then the readings were converted to an actual volume (μl) by reference to the standard curve. Upon collection, the samples were immediately stored at -80°C before lyophilization, which was performed by freeze-drying under vacuum. For laboratory analysis, 200 μl of phosphate buffered saline (pH 7.2) was used to re-elute the samples. The tubes were shaken gently for 1 min. and then centrifuged at 2000 *g* for 15 min. at 4°C, before being processed on the enzyme-linked immunosorbent assay (ELISA) plate.

Quantification of RANKL and OPG concentrations in GCF

The total amount of RANKL and OPG in the GCF samples was determined by human-specific ELISA in accordance with the manufacturer's instructions (total sRANKL ELISA kit: Immundiagnostik AG, Bensheim, Germany, and Osteoprotegerin ELISA kit: Biomedica, Vienna, Austria). These assays measure the total levels of RANKL or OPG present in the GCF, including the unbound forms, as well as the RANKL–OPG complex. Calculation of the RANKL and OPG concentration in each GCF sample was performed by dividing the total amount of RANKL or OPG by the volume of GCF [RANKL or OPG concentration (pg/μl) = total RANKL or OPG (pg)/GCF volume (μl)].

Statistical analysis

Statistical analysis was performed using the repeated measures analysis of variance, and the Bonferroni post hoc test to compare differences between groups. In order to investigate the correlations between RANKL, OPG and RANKL/OPG ratio levels in GCF and clinical parameters, Spearman's rank correlation analysis was used. Differences were considered statistically significant at $p < 0.01$.

Results

Clinical findings of at baseline and after treatment

The full-mouth and site-specific clinical data for the CP and G-AgP groups are shown in Table 1. After treatment, both CP and G-AgP groups exhibited significant improvements of all clinical parameters measures, at all follow-up time-points (2, 3, 4 months), compared with baseline.

Changes of RANKL levels after treatment

In the CP group, when the total amount of RANKL was considered, there were no changes over time after treatment (Fig. 1a). However, the concentration of RANKL was increased from 433 ± 269 pg/μl at baseline, to 1212 ± 862 pg/μl after 2 months, 931 ± 582 pg/μl after 3 months and 829 ± 412 pg/μl after 4 months (Fig. 1b). This increase proved to be significant ($p < 0.01$) only at 2 months post-treatment. In the G-AgP group, the total amount of RANKL was not altered up to 3 months post-treatment, but this was significantly ($p < 0.01$) increased after 4 months (Fig. 1a). Accordingly, the concentration of RANKL was increased from 468 ± 580 pg/μl at baseline, to 924 ± 434 pg/μl after 2 months, 975 ± 653 pg/μl after 3 months and 1313 ± 620 pg/μl after 4 months, but only the latest proved to be statistically significant ($p < 0.001$) (Fig. 1b), similar to the total amount of RANKL. When RANKL levels were compared between CP and G-AgP groups at the various time-points, there were no statistically significant differences.

Changes of OPG levels after treatment

In the CP group, neither the total amounts (Fig. 2a) nor the concentration levels (Fig. 2b) of OPG in GCF changed significantly over the post-treatment period, compared with baseline levels. In the case of G-AgP, the total amount of OPG in GCF did not change over the 4-month period after treatment (Fig. 2a). However, the concentration of OPG was increased from 71 ± 74 pg/μl at baseline, to 147 ± 62 pg/μl after 2 months, 148 ± 91 pg/μl after 3 months and 207 ± 105 pg/μl after 4 months (Fig. 2b). The latest increase (4 months), proved to be statistically significant, as in the case of RANKL concentration. Interestingly, OPG concentration levels in the CP group proved to be significantly ($p < 0.01$) higher than the G-AgP group at 4 months post-treatment (Fig. 2b).

Table 1. Clinical parameters before and after treatment (mean ± standard deviation)

Full-mouth	PPD (mm)	CAL (mm)	BOP (%)	PI (%)
A. Chronic periodontitis ($n = 14$ subjects)				
Baseline	3.82 ± 0.56	5.32 ± 1.00	65.6 ± 17.8	88.4 ± 20.7
2 months	2.38 ± 0.33*	3.97 ± 0.64*	18.5 ± 7.8*	34.8 ± 11.7*
3 months	2.33 ± 0.28*	3.87 ± 0.62*	17.7 ± 8.0*	29.6 ± 7.5*
4 months	2.15 ± 0.24*	3.79 ± 0.62*	15.3 ± 6.3*	26.5 ± 3.5*

Site specific	PPD (mm)	CAL (mm)	PBI	PI	GCF (μl)
Baseline	5.98 ± 1.14	7.14 ± 1.83	2.0 ± 1.8	2.8 ± 0.9	0.31 ± 0.22
2 months	2.50 ± 0.51*	4.21 ± 1.18*	0.2 ± 0.4*	0.8 ± 0.7*	0.14 ± 0.11*
3 months	2.28 ± 0.46*	3.92 ± 1.07*	0.2 ± 0.4*	0.6 ± 0.6	0.15 ± 0.07*
4 months	2.28 ± 0.46*	4.28 ± 1.13*	0.2 ± 0.4*	0.5 ± 0.6	0.10 ± 0.06*

Full-mouth	PPD (mm)	CAL (mm)	BOP (%)	PI (%)
B. Generalized aggressive periodontitis ($n = 13$ subjects)				
Baseline	4.28 ± 0.73	5.15 ± 0.67	80.3 ± 17.2	93.8 ± 8.7
2 months	2.45 ± 0.25*	3.57 ± 0.57*	22.9 ± 7.4*	35.8 ± 13.5*
3 months	2.39 ± 0.26*	3.46 ± 0.64*	24.3 ± 13.8*	36.6 ± 12.4*
4 months	2.21 ± 0.20*	3.39 ± 0.63*	21.7 ± 9.2*	28.0 ± 4.9*

Site specific	PPD (mm)	CAL (mm)	PBI	PI	GCF (μl)
Baseline	7.30 ± 1.37	7.92 ± 1.44	2.3 ± 0.8	2.5 ± 1.0	0.40 ± 0.35
2 months	3.23 ± 0.92*	4.76 ± 0.92*	0.4 ± 0.6*	0.7 ± 1.0*	0.12 ± 0.05*
3 months	3.07 ± 0.95*	4.53 ± 1.26*	0.4 ± 0.6*	0.6 ± 0.6*	0.13 ± 0.08*
4 months	2.84 ± 0.80*	4.30 ± 1.03*	0.3 ± 0.5*	0.3 ± 0.4*	0.10 ± 0.06*

*Significant difference from baseline ($p < 0.001$).

BOP, bleeding on probing; CAL, clinical attachment levels; GCF, gingival crevicular fluid volume; PBI, papilla bleeding index; PI, plaque index; PPD, probing pocket depth.

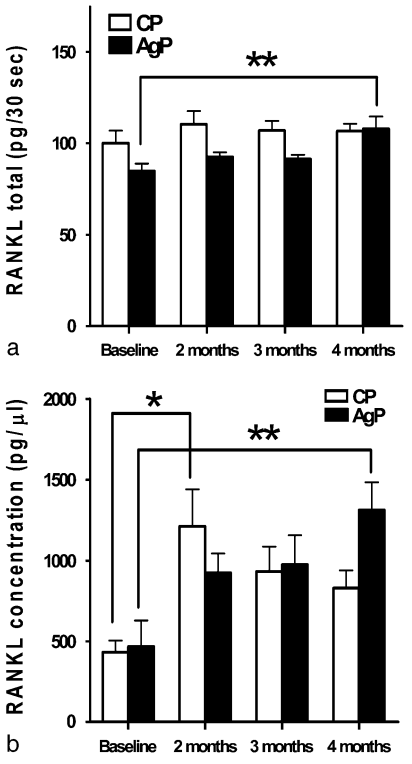

Fig. 1. Receptor activator of NF-κB ligand (RANKL) total amounts (a) and concentrations (b) in gingival crevicular fluid from chronic periodontitis (CP; $n = 14$) and generalized aggressive periodontitis (G-AgP; $n = 13$) patients, at baseline, 2, 3 and 4 months after treatment. The bars represent the mean ± standard error of mean in each group and time-point. The asterisks indicate statistically significant differences between groups ($^{*}p < 0.01$, $^{**}p < 0.001$).

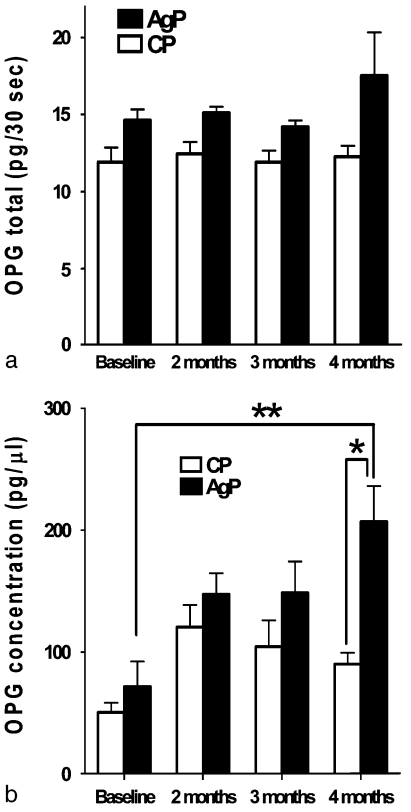

Fig. 2. Osteoprotegerin (OPG) total amounts (a) and concentrations (b) in gingival crevicular fluid from chronic periodontitis (CP; $n = 14$) and generalized aggressive periodontitis (G-AgP; $n = 13$) patients, at baseline, 2, 3 and 4 months after treatment. The bars represent the mean ± standard error of mean in each group and time-point. The asterisks indicate statistically significant differences between groups ($^{*}p < 0.01$, $^{**}p < 0.001$).

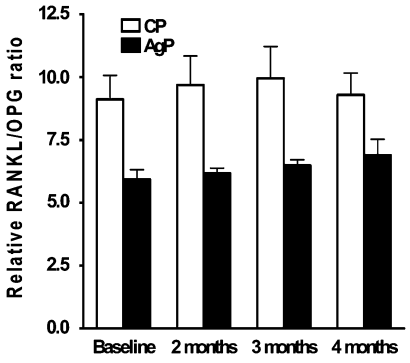

Fig. 3. Receptor activator of NF-κB ligand/osteoprotegerin (RANKL/OPG) ratio in gingival crevicular fluid from chronic periodontitis (CP; $n = 14$) and generalized aggressive periodontitis (G-AgP; $n = 13$) patients, at baseline, 2, 3 and 4 months after treatment. The bars represent the mean standard error of mean in each group and time-point. No statistically significant differences were detected between patient groups or time-points.

Table 2. Correlations between clinical parameters and total amounts of RANKL, OPG and relative RANKL/OPG ratio

Clinical parameters	RANKL	OPG	RANKL/OPG
A. Chronic periodontitis			
PPD	0.004	−0.007	0.061
CAL	−0.306*	0.181	−0.262
PBI	−0.041	−0.228	0.110
PI	−0.092	−0.119	0.003
B. Generalized aggressive periodontitis			
PPD	−0.233	−0.001	−0.202
CAL	−0.130	−0.073	−0.132
PBI	−0.408*	−0.038	−0.363*
PI	−0.195	−0.098	−0.071

The *r* value is provided.
Spearman's rank correlation analysis was used.
$^{*}p < 0.01$.
CAL, clinical attachment loss; OPG, osteoprotegerin; PI, plaque index; PBI, papilla bleeding index; PPD, probing pocket depth; RANKL; receptor activator of NF-κB ligand.

Changes in the RANKL/OPG ratio levels after treatment

The relative RANKL/OPG ratio was also calculated, based on the previously obtained individual RANKL and OPG total amounts, for both CP and G-AgP groups. Interestingly, this ratio maintained unchanged from baseline and throughout the post-treatment follow-up period, in both CP and G-AgP groups (Fig. 3). In addition, there were no statistically significant differences between CP and G-AgP groups at any of the time-points.

Correlation of RANKL and OPG and relative ratio with clinical parameters

The correlations of clinical parameters with RANKL and OPG total amounts as well as their relative ratio were investigated by Spearman's rank correlation analysis (Table 2). The total amounts were chosen over the concentrations for the correlation analyses, in order to eliminate the effect of GCF volume. In the CP group, RANKL total amount was negatively correlated with CAL, whereas in the G-AgP group this was negatively correlated with PBI.

Discussion

The RANKL–OPG system has an important role in the pathogenesis of periodontal disease. An increased RANKL/OPG ratio is considered indicative of the occurrence of untreated periodontitis and it is suggested that it could potentially serve as a molecular diagnostic marker for the disease (Mogi et al. 2004, Bostanci et al. 2007, Taubman et al. 2007). The present study was undertaken with the aim to monitor quantitative changes of the RANKL–OPG system in GCF, in response to non-surgical periodontal treatment, over a period of 4 months.

The data indicate that periodontal treatment does not affect RANKL or OPG total amounts in CP patients. Hence, conventional therapy alone cannot modify the overall capacity of the tissue to produce these factors. When their concentration in GCF was considered, there was a transient increase of RANKL and OPG levels at 2 months post-treatment, which proved to be statistically significant in the case of

RANKL. This is likely a result of the reduced local inflammation, and subsequently of the lower GCF sampling volume. Increased RANKL and OPG GCF concentrations after periodontal treatment have been reported previously in a cohort of patients with diabetes mellitus (Santos et al. 2010). Moreover, another study reported no changes in RANKL, but a decrease in OPG GCF levels, 4 weeks after initial periodontal treatment (Buduneli et al. 2009). In the present study, the RANKL/OPG ratio also remained unchanged despite the improved clinical treatment outcomes. A slight increase of the RANKL/OPG expression ratio after non-surgical periodontal treatment has been reported previously in CP patients (Dereka et al. 2010). Hence, collectively from the present and previous studies on CP (Buduneli et al. 2009, Dereka et al. 2010, Santos et al. 2010), it can be deduced that a clinically successful treatment outcome may not predictably result in reduction of the RANKL/OPG ratio.

It was of further interest to investigate changes in RANKL and OPG levels after treatment, in patients with G-AgP. As it is debatable whether CP and G-AgP constitute different diseases, or different forms of the same disease (Armitage & Cullinan 2010, Armitage et al. 2010), it is anticipated that any differential responses to periodontal treatment could contribute to our understanding of this issue. It was found that periodontal treatment did not alter significantly RANKL and OPG GCF concentrations over a 3-month period. Although at 4 months after treatment, both RANKL and OPG levels were significantly elevated, their relative RANKL/OPG ratio maintained unchanged throughout the whole monitoring period. A potentially interesting finding is the negative correlation between RANKL or the RANKL/OPG ratio and PBI, which may indicate that reduction of inflammation is not necessarily associated with a reduced capacity for bone destruction in G-AgP. An interesting finding is that when OPG levels were compared between the two patient groups, these were higher in CP compared with G-AgP after 4 months of treatment, which may indicate different healing patterns. Hence, temporal differences may exist between CP and G-AgP in the individual RANKL and OPG responses after treatment, but the overall common feature is that the relative RANKL/OPG ratio is not affected in either form of the disease, despite the clinical improvements.

This finding does not devalue the RANKL/OPG ratio as a potential diagnostic marker for periodontitis (Bostanci et al. 2007), as it could still indicate sites with prior disease occurrence at the molecular level. However, the present experimental design does not take under consideration prior confirmed activity of the sampled lesions. Studies using the tolerance method (Haffajee et al. 1983) to confirm periodontal disease progression, have indicated higher RANKL GCF (Silva et al. 2008) and tissue gene expression (Dutzan et al. 2009) levels in active sites, compared with inactive ones. In another study involving CP patients, it was reported that sites with persistent bleeding on probing after periodontal therapy exhibit higher RANKL than OPG gingival tissue expression (Menezes et al. 2008).

In conclusion, the present study indicates that, despite its potential diagnostic value, the RANKL/OPG ratio may not be predictive of clinical improvements of the periodontal status, as it remains unchanged after conventional periodontal treatment. A persistently high RANKL/OPG ratio even after a clinically successful treatment outcome could indicate that tissue healing may not be commensurate with the elimination of molecular mechanisms for bone resorption, and thus a risk for further periodontal tissue breakdown may still exist. Nevertheless, such a question would need to be addressed in longitudinal studies of prognostic nature, with higher statistical power and potentially taking under consideration prior disease progression of the treated sites. Moreover, the persistently high RANKL/OPG ratio after initial periodontal treatment may indicate the need for adjunctive host response modulation therapies for periodontal disease management (Salvi & Lang 2005). Such therapies could target specifically the RANKL–OPG system, thus preventing or slowing down further bone loss. A recently introduced pharmacological intervention that serves in part this purpose is the per os administration of omega-3 fatty acids (El-Sharkawy et al. 2010). Alternatively, the effectiveness of Demosunab, a novel monoclonal antibody against RANKL used in the treatment of bone-destructive disorders (Rizzoli et al. 2010), needs to be tested as an adjunctive treatment for periodontitis as well.

References

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Armitage, G. C. & Cullinan, M. P. (2010) Comparison of the clinical features of chronic and aggressive periodontitis. *Periodontology 2000* **53**, 12–27.

Armitage, G. C., Cullinan, M. P. & Seymour, G. J. (2010) Comparative biology of chronic and aggressive periodontitis: introduction. *Periodontology 2000* **53**, 7–11.

Bartold, P. M., Cantley, M. D. & Haynes, D. R. (2010) Mechanisms and control of pathologic bone loss in periodontitis. *Periodontology 2000* **53**, 55–69.

Bostanci, N., Ilgenli, T., Emingil, G., Afacan, B., Han, B., Toz, H., Atilla, G., Hughes, F. J. & Belibasakis, G. N. (2007) Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *Journal of Clinical Periodontology* **34**, 370–376.

Buduneli, N., Buduneli, E. & Kutukculer, N. (2009) Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. *Journal of Periodontology* **80**, 1274–1280.

Dereka, X. E., Markopoulou, C. E., Fanourakis, G., Tseleni-Balafouta, S. & Vrotsos, I. A. (2010) RANKL and OPG mRNA level after non-surgical periodontal treatment. *Inflammation* **33**, 200–206.

Dutzan, N., Gamonal, J., Silva, A., Sanz, M. & Vernal, R. (2009) Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-kappa B ligand, interleukin (IL)-17, IL-10 and transforming growth factor-beta during the progression of chronic periodontitis. *Journal of Clinical Periodontology* **36**, 396–403.

El-Sharkawy, H., Aboelsaad, N., Eliwa, M., Darweesh, M., Alshahat, M., Kantarci, A., Hasturk, H. & Van Dyke, T. E. (2010) Adjunctive treatment of chronic periodontitis with daily dietary supplementation with omega-3 Fatty acids and low-dose aspirin. *Journal of Periodontology* **81**, 1635–1643.

Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1983) Comparison of different data analyses for detecting changes in attachment level. *Journal of Clinical Periodontology* **10**, 298–310.

Lacey, D. L., Timms, E., Tan, H. L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J. & Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* **93**, 165–176.

Lerner, U. H. (2006) Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. *Journal of Dental Research* **85**, 596–607.

Liu, Y. C., Lerner, U. H. & Teng, Y. T. (2010) Cytokine responses against periodontal infection: protective and destructive roles. *Periodontology 2000* **52**, 163–206.

Lu, H. K., Chen, Y. L., Chang, H. C., Li, C. L. & Kuo, M. Y. (2006) Identification of the osteoprotegerin/receptor activator of nuclear factor-kappa B ligand system in gingival crevicular fluid and tissue of patients with chronic periodontitis. *Journal of Periodontal Research* **41**, 354–360.

Menezes, R., Garlet, T. P., Letra, A., Bramante, C. M., Campanelli, A. P., Figueira Rde, C., Sogayar, M. C., Granjeiro, J. M. & Garlet, G. P. (2008) Differential patterns of receptor activator of nuclear factor kappa B ligand/osteoprotegerin expression in human periapical granulomas: possible association with progressive or stable nature of the lesions. *Journal of Endodontics* **34**, 932–938.

Mogi, M., Otogoto, J., Ota, N. & Togari, A. (2004) Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *Journal of Dental Research* **83**, 166–169.

Rizzoli, R., Yasothan, U. & Kirkpatrick, P. (2010) Denosumab. *Nature Reviews Drug Discovery* **9**, 591–592.

Salvi, G. E. & Lang, N. P. (2005) Host response modulation in the management of periodontal diseases. *Journal of Clinical Periodontology* **32** (Suppl. 6), 108–129.

Santos, V. R., Lima, J. A., Goncalves, T. E., Bastos, M. F., Figueiredo, L. C., Shibli, J. A. & Duarte, P. M. (2010) Receptor activator of NF-(capital KA, Cyrillic)B ligand/osteoprotegerin ratio in sites of chronic periodontitis of poorly-controlled and well-controlled type 2 diabetic subjects. *Journal of Periodontology* **81**, 1455–1465.

Silva, N., Dutzan, N., Hernandez, M., Dezerega, A., Rivera, O., Aguillon, J. C., Aravena, O., Lastres, P., Pozo, P., Vernal, R. & Gamonal, J. (2008) Characterization of progressive periodontal lesions in chronic periodontitis patients: levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *Journal of Clinical Periodontology* **35**, 206–214.

Simonet, W. S., Lacey, D. L., Dunstan, C. R., Kelley, M., Chang, M. S., Luthy, R., Nguyen, H. Q., Wooden, S., Bennett, L., Boone, T., Shimamoto, G., DeRose, M., Elliott, R., Colombero, A., Tan, H. L., Trail, G., Sullivan, J., Davy, E., Bucay, N., Renshaw-Gegg, L., Hughes, T. M., Hill, D., Pattison, W., Campbell, P., Sander, S., Van, G., Tarpley, J., Derby, P., Lee, R. & Boyle, W. J. (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* **89**, 309–319.

Tang, T. H., Fitzsimmons, T. R. & Bartold, P. M. (2009) Effect of smoking on concentrations of receptor activator of nuclear factor kappa B ligand and osteoprotegerin in human gingival crevicular fluid. *Journal of Clinical Periodontology* **36**, 713–718.

Taubman, M. A., Kawai, T. & Han, X. (2007) The new concept of periodontal disease pathogenesis requires new and novel therapeutic strategies. *Journal of Clinical Periodontology* **34**, 367–369.

Teitelbaum, S. L. & Ross, F. P. (2003) Genetic regulation of osteoclast development and function. *Nature Reviews Genetics* **4**, 638–649.

Vernal, R., Chaparro, A., Graumann, R., Puente, J., Valenzuela, M. A. & Gamonal, J. (2004) Levels of cytokine receptor activator of nuclear factor kappaB ligand in gingival crevicular fluid in untreated chronic periodontitis patients. *Journal of Periodontology* **75**, 1586–1591.

Address:
Nagihan Bostanci
Center of Dental Medicine
Institute of Oral Biology
University of Zürich
Plattenstrasse 11
8032 Zürich
Switzerland
E-mail: nagihan.bostanci@zzm.uzh.ch

Clinical Relevance

Scientific rationale for study: RANKL induces, whereas OPG inhibits bone resorption. An increased RANKL/OPG ratio in GCF is indicative of the occurrence periodontitis. This study investigated if this ratio is predictably reduced in GCF after non-surgical periodontal treatment.

Principal findings: The RANKL/OPG ratio in GCF was not affected by treatment, despite the improved clinical outcomes over 4 months of monitoring, in both CP and G-AgP.

Practical implications: The RANKL/OPG ratio may not be a helpful molecular predictor of clinically successful treatment. As conventional therapy does not negatively modulate this ratio, the host could still be susceptible to further bone loss. Adjunctive treatments targeting RANKL–OPG may be useful in this respect.