J Clin Periodontol 2006; 33: 302–308 doi: 10.1111/j.1600-051X.2006.00904.x

Journal of Clinical Periodontology

Cathepsin K levels in the crevicular fluid of dental implants: a pilot study

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Strbac GD, Monov G, Cei S, Kandler B, Watzek G, Gruber R. Cathepsin K levels in the crevicular fluid of dental implants: a pilot study. J Clin Periodontol 2006; 33: 302–308. doi: 10.1111/j.1600-051X.2006.00904.x. © Blackwell Munksgaard, 2006.

Abstract

Objective: To determine the concentration of cathepsin K secreted into the crevicular fluid around dental implants and its correlation with clinical parameters of healthy implants and implants showing clinical signs of peri-implantitis.

Material and Methods: Nineteen patients with 40 implants with and without peri-implantitis were enrolled in the study. Peri-implantitis was diagnosed by the pocket probing depth (PD), the modified bleeding index (MBI), the modified plaque index (MPI) and by radiographic signs of bone loss. Gingival crevicular fluid collected from the buccal and lingual sites was adsorbed to filter strips. Cathepsin K levels and total protein within the crevicular fluid were determined by immunoassay and the bicinchoninic method, respectively.

Results: Cathepsin K per filter strip normalized to the time of collection was 10.1 (0–33.5) pmol/sample around control implants and 22.4 (3.7–56.3) pmol/sample in the peri-implantitis group. The difference between the medians was significant ($p < 0.01$). Absolute cathepsin K levels in the crevicular fluid of all implants investigated showed a positive correlation with PD ($R = 0.25$; $p = 0.03$), MPI ($R = 0.28$; $p = 0.01$) and MBI ($R = 0.32$; $p < 0.01$). Absolute cathepsin K levels in the crevicular fluid also correlated with the adsorbed volume of gingival crevicular fluid ($R = 0.51$; $p < 0.01$). When normalized to the adsorbed volume of gingival crevicular fluid, the concentration of cathepsin K was 2.2 (0.01–6.4) nM around control implants and 1.7 (0.4–4.6) nM in the peri-implantitis group ($p = 0.33$). Patients' age correlated with sample volume and with cathepsin K normalized to the adsorbed volume of gingival crevicular fluid ($R = 0.39$; $p < 0.01$). Moreover, significant differences between male and female ($p < 0.01$, $p < 0.01$), and between mandible and maxilla ($p < 0.05$, $p < 0.01$), but not between buccal and lingual sites ($p = 0.99$, $p = 0.93$), were observed when analysed for the parameters adsorbed volume and absolute cathepsin K levels.

Conclusion: Clinical parameters of peri-implantitis are associated with a higher amount of cathepsin K and a higher volume adsorbed to filters strips. To establish cathepsin K as a biochemical parameter to monitor peri-implant tissue health, age, sex and collection site should be considered to avoid interfering influences because of sample inhomogenity. Also a prospective study over time including more patients would be necessary.

Key words: bone resorption; cathepsin K; crevicular fluid; immunoassay; peri-implantitis

Accepted for publication 9 January 2006

Peri-implantitis is among the main causes of late implant failure. Its incidence was reported to be 8.6% within 5 years (Kourtis et al. 2004, Pjetursson et al. 2004). Its aetiology is linked to persisting bacterial contamination and to functional overloading that may lead to chronic inflammation (Mombelli et al. 1987, Tonetti & Schmid 1994, De Smet et al. 2001). Peri-implant mucositis may progress to peri-implantitis, which is characterized by rapid bone loss within the early stages of the disease (Marinello et al. 1995, Tillmanns et al. 1997, Zitzmann et al. 2004). Clinical parameters, e.g. pocket probing depth (PD), modified bleeding index (MBI) and modified plaque index (MPI), as

well as radiographic signs of bone loss have been used for the diagnosis and monitoring of disease progression (Mombelli & Lang 1994, Esposito et al. 1998, Salvi & Lang 2004). Clinical parameters and radiographs are, however, historical determinants and provide limited information about the dynamic pathophysiological mechanisms of disease initiation and progression. Therefore, simple and reliable clinical tests are needed to monitor peri-implant tissue health during recall visits (Mombelli & Lang 1994).

Biochemical parameters within the peri-implant crevicular fluid provide insights into the microenvironment around dental implants, and thus can serve as prognostic markers for monitoring disease progression and the effects of therapeutic interventions. Peri-implant crevicular fluid contains cytokines, growth factors, enzymes and their cleavage products, which are released by cells of soft and mineralized tissue, depending on the disease state (Oringer et al. 1998, Ataoglu et al. 2002, Murata et al. 2002, Ma et al. 2003, Kivela-Rajamaki et al. 2003a, b, Liskmann et al. 2004). Increased levels of inflammatory cytokines at sites of peri-implantitis can stimulate the formation and activation of bone resorbing osteoclasts (Lam et al. 2000, Wei et al. 2005) and therefore contribute to the initiation and progression of bone resorption.

Osteoclasts are the exclusive bone resorbing cells originating from progenitor cells of the monocyte/macrophage lineage (Chambers 2000, Boyle et al. 2003, Teitelbaum & Ross 2003). Osteoclastogenesis is a highly regulated multistep process involving the immigration, proliferation, differentiation, fusion and activation of progenitor cells. This process is coordinated by the interaction of three members of the tumor necrosis factor superfamily: receptor activator of NF-κB ligand (RANKL), RANK and osteoprotegerin (Suda et al. 1999). Mature osteoclasts are characterized by a sealing zone, which allows their intimate attachment to the bone surface. As a result, an extracellular lysosomal compartment is formed (Teitelbaum 2000). Osteoclasts acidify the lysosomal compartment and release proteases such as cathepsin K and matrix metalloproteinase 9, also called gelatinase B, to digest the non-mineralized bone matrix (Teitelbaum 2000). The gelatinase B activity is increased in crevicular fluid at peri-implantitis sites (Ma et al. 2003). Other members of the family of cathepsin proteases, such as cathepsin B, C, D, G and L, were detected in the healthy oral tissue and in the crevicular fluid of periodontal disease (Trabandt et al. 1995, Tervahartiala et al. 1996, Chen et al. 1998, Buchmann et al. 2002, Dickinson 2002, Soell et al. 2002). Data on cathepsin K levels in the crevicular fluid from peri-implantitis have so far not been available.

Cathepsin K is a cysteine protease highly expressed by osteoclasts (Rantakokko et al. 1996). Lung tissue, breast cancer and giant cell tumors also produce detectable amounts of the enzyme (Littlewood-Evans et al. 1997, Buhling et al. 2000, Lindeman et al. 2004). In the dental area, cathepsin K is expressed by odontoclasts after experimental tooth movement and in the jaw bones of mice (Tsuji et al. 2001, Ida-Yonemochi et al. 2004). Cathepsin K is synthesized as a zymogene, but the osteoclasts apposed to the bone surface almost exclusively release the active form (Dodds et al. 2001, Rieman et al. 2001). Activated cathepsin K degrades bone matrix proteins including type I collagen, osteopontin and osteonectin at a low pH (Bossard et al. 1996). Overexpression of cathepsin K in transgenic mouse models leads to high-turnover bone loss (Kiviranta et al. 2001), whereas dysfunction mutations of the cathepsin K gene show the bone phenotype of osteopetrosis in mice (Saftig et al. 1998, Gowen et al. 1999) and pycnodysostosis in men (Nishi et al. 1999). Cathepsin K is a potential therapeutic target in the treatment of osteoporosis (Yamashita & Dodds 2000, Wang et al. 2004). Chronic inflammation as in rheumatoid arthritis is associated with increased serum levels of cathepsin K (Skoumal et al. 2005). In this pilot study, cathepsin K levels were determined in the crevicular fluid around dental implants and correlated with clinical parameters of peri-implantitis.

Material and Methods

Study population

Forty implants of 13 female and six male subjects from the Department of Oral Surgery, Medical University of Vienna, were included in the study. The mean age of the subjects was 61 (30–84) years. As indicated in Table 3A, a total of 56 samples (36 control/20 peri-implantitis) from females and 24 samples (four control/20 peri-implantitis) from males were included. Fifty-four samples were taken from the mandible (24 control/30 peri-implantitis) and 26 from the maxilla (16 control/10 peri-implantitis) (Table 3A). Thirteen subjects had at least one implant with clinical and radiographic signs of peri-implantitis. Dental implants were under functional loading for at least 1 year. None of the subjects had a history of systemic disease. None of them had been on systemic antibiotics during the last 6 months. All patients were non-smokers. Written informed consent was obtained from each subject. The protocol was approved by the ethical committee of the Medical University of Vienna (EK#259/2004).

Collection of peri-implant crevicular fluid

Peri-implant crevicular fluid was collected with the intra-crevicular method (Griffiths 2003). Implant surfaces were air dried and isolated by cotton rolls. Paper strips (Periopaper, ProFlow, Amittyville, NY, USA) were inserted into the base of the implant pocket for 30 s. Samples were collected from the lingual and buccal aspects of each implant. The adsorbed volume was determined by impedance measurement based on a calibration curve (Periotron 8000, Oralflow Inc., Plainview, NY, USA). The filter strips were placed in 1.5 ml plastic tubes containing 400 μl of phosphate-buffered saline. The samples were stored at -20°C before analysis.

Evaluation of the peri-implant status by clinical parameters and radiographs

A full-oral examination was performed according to defined parameters (Mombelli et al. 1987, Mombelli & Lang 1994, Esposito et al. 1998, Salvi & Lang 2004). The PD was measured as the linear distance from the free mucosal margin to the bottom of the pocket at a probing force of 0.25 N (PD, PCP 12 periodontal probe; Hu-Friedy, Chicago, IL, USA). The bleeding tendency of the marginal peri-implant tissue was evaluated using the MBI (score 0, no bleeding when periodontal probe is passed along the gingival margin adjacent to the implant; score 1, isolated bleeding spots visible; score 2: blood forms a confluent red line on margin; score 3, heavy or profuse bleeding). Plaque accumulation was evaluated by the MPI (score 0, no

detection of plaque; score 1, plaque recognized only by running a probe across a smooth marginal surface of the implant; score 2, plaque can be seen at a glance; score 3, abundance of soft matter). All measurements were performed buccal, lingual, mesial and distal to each implant, and means were used for statistical analysis. Peri-implantitis was considered to be positive when the following parameters were exceeded: PD > 3.00 mm, MBI > 1.00 and MPI ⩾ 1.00. Radiographic loss of peri-implantitis supporting bone was considered positive when exposed implant threads were visible.

Determination of cathepsin K and total protein

Cathepsin K was eluted from the filter strips by continuous shaking for 30 min followed by 5 min centrifugation at $10\,000 \times g$. The immunoassay based on the sandwich technique was performed following the instructions of the manufacturer (Biomedica, Vienna, Austria). The antibodies recognize positions 1–20 and 196–210 of the zymogen, and thus also the active enzyme. The assay does not cross-react with structurally related cathepsin E, D, B or L or with rheumatoid factors. The manufacturer sets the sensitivity of the assay at 1.1 pmol/l. The intra-assay variations range from 4% to 8%. Cathepsin K in serum is stable at 4°C for 2 days and can be stored frozen at less than −20°C for long periods of time. Total protein in the eluted samples was determined with the bicinchoninic method (Pierce Chemical Co., Rockford, IL, USA). Absolute amounts of cathepsin K per time of collection (pmol cathepsin K eluted per filter strip), concentrations per time of collection (pmol cathepsin K eluted per filter strip and normalized to the adsorbed volume of crevicular fluid) and relative amounts per time of collection (pmol cathepsin K per filter strip normalized to the adsorbed total protein) were recorded. Kinetic assays were not performed because cathepsin K is inactivated after 1 h at pH 7.5 and 37°C (Dickinson 2002).

Statistical analysis

Median cathepsin K levels in the peri-implantitis and control groups were compared with the Mann–Whitney test. Regression analysis was performed to correlate cathepsin K levels with the clinical parameters, i.e. PD, MBI and MPI. Absolute amounts of cathepsin K per time of collection were correlated with the adsorbed volumes to filter strips and with the total eluted protein. Analyses were performed with Statgraphics plus for Windows (Statistical Graphics Corp., Englewood Cliffs, NJ, USA). Significance was assigned at the 5% level.

Results

Cathepsin K levels in crevicular fluid of peri-implantitis

Cathepsin K per filter strip normalized to the time of collection was 10.1 (0–33.5) pmol/sample around control implants and 22.4 (3.7–56.3) pmol/sample in the peri-implantitis group. The difference between the medians was significant ($p < 0.01$). Normalized to the adsorbed volume of the gingival crevicular fluid, the median concentration was 2.2 (0.01–6.4) nM around control implants and 1.7 (0.4–4.6) nM in peri-implantitis ($p = 0.33$). Cathepsin K normalized to protein within the gingival crevicular fluid reached 0.07 (0–2.4) nmol/μg protein around control implants and 0.09 (0.01–2.5) nmol/μg protein in the peri-implantitis group ($p = 0.22$) (Fig. 1).

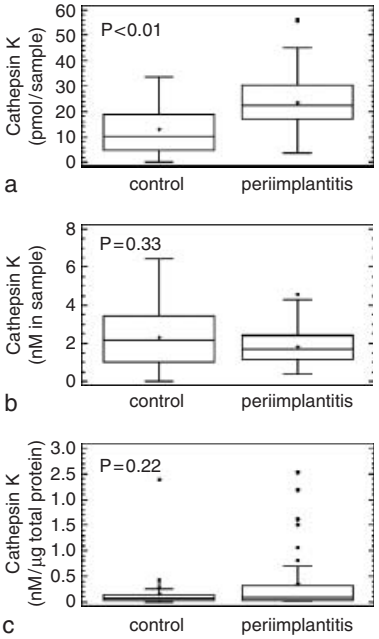

Fig. 1. Cathepsin K values in crevicular fluid of implants with and without peri-implantitis. Cathepsin K levels were determined within the crevicular fluid of implants with and without peri-implantitis. Peri-implantitis was defined by clinical parameters and by qualitative radiographic evaluation. Samples were collected from the lingual and buccal sites of each implant. (a) Absolute amount of cathepsin K per filter strip normalized to the time of collection, (b) cathepsin K normalized to the adsorbed volume of the gingival crevicular fluid and (c) cathepsin K normalized to protein within the gingival crevicular fluid. The difference between the two groups was significant (Mann–Whitney test; $p < 0.05$).

Cathepsin K levels negatively correlate with clinical parameters

(A) Absolute cathepsin K levels in the crevicular fluid of all implants investigated showed a positive correlation with the clinical parameters, i.e. PD ($R = 0.25$; $p = 0.03$), MPI ($R = 0.28$; $p = 0.01$) and MBI ($R = 0.32$; $p < 0.01$). A positive correlation was also detected with the adsorbed volume of gingival crevicular fluid ($R = 0.51$; $p < 0.01$) but not with adsorbed total protein ($R = 0.02$; $p = 0.84$). (B) There was a statistically significant relation between the cathepsin K concentration normalized to the sample volume and the clinical parameters PD, MPI and MBI. The correlation coefficient R equalled −0.27 (PD), −0.27 (MPI) and −0.20 (MBI). The corresponding p-values of 0.02 (PD), 0.02 (MPI) and 0.07 (MBI) indicate a weak negative relation between the variables. (C) Cathepsin K values normalized to protein adsorbed to filter strips did not correlate with any of the investigated clinical parameters. The correlation coefficient R was −0.05 ($p = 0.67$) for PD, −0.15 ($p = 0.19$) for MPI and −0.04 ($p = 0.72$) for MBI (Fig. 2).

Sample volume but not total protein correlates with cathepsin K values

(A) Regression analysis with volume as the independent variable and cathepsin K as the dependent variable showed a moderately strong correlation with the variables at $R = 0.51$ ($p < 0.01$). (B) Total protein per filter strip did not correlate with the total amount of cathepsin K ($R = -0.02$; $p = 0.84$) (Fig. 3).

Clinical parameters show a strong inter-related correlation

Correlation coefficients with PD as the dependent variable and MBI and MPI as the independent variables were 0.82 and 0.66, respectively. With MBI as dependent

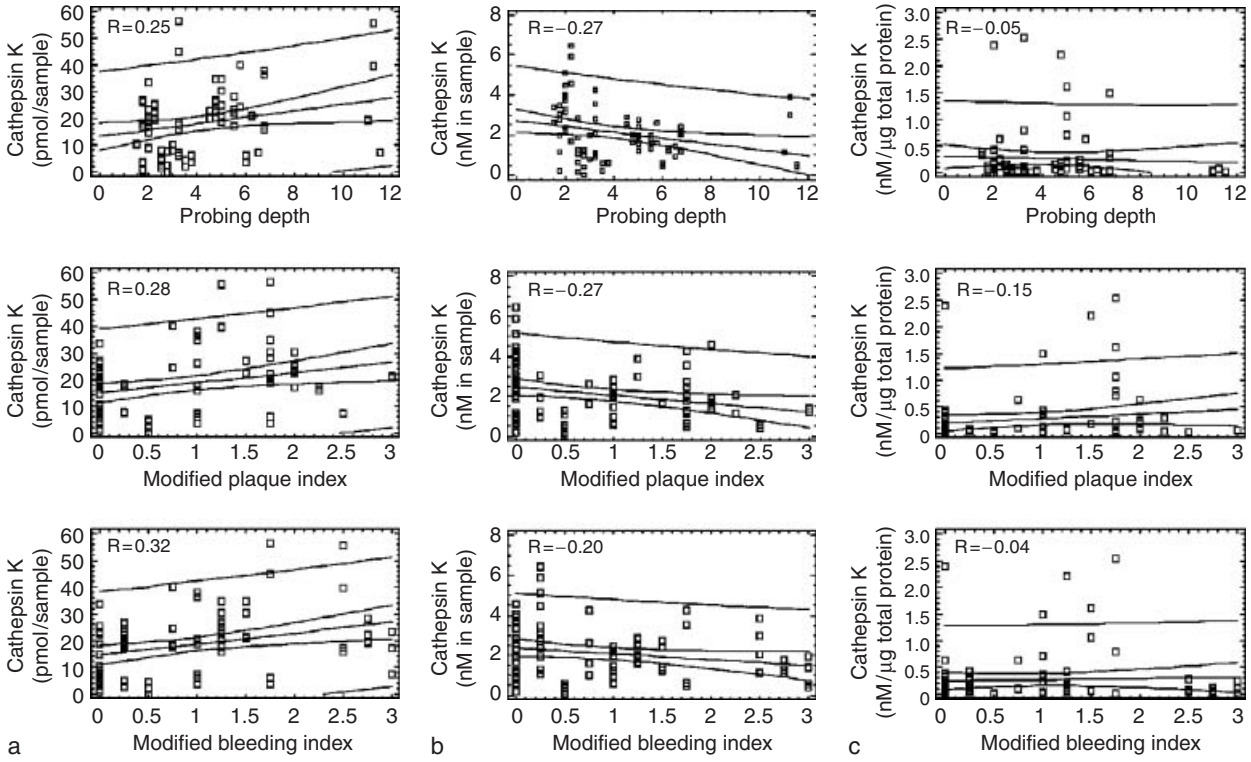

Fig. 2. Correlation of cathepsin K with clinical parameters of peri-implantitis. (a) Absolute amount of cathepsin K per filter strip normalized to the time of collection, (b) cathepsin K normalized to the adsorbed volume of the gingival crevicular fluid and (c) cathepsin K normalized to protein within the gingival crevicular fluid *versus* clinical parameters, i.e. pocket probing depth (PD), modified bleeding index (MBI) and modified plaque index (MPI). *R* = correlation coefficient.

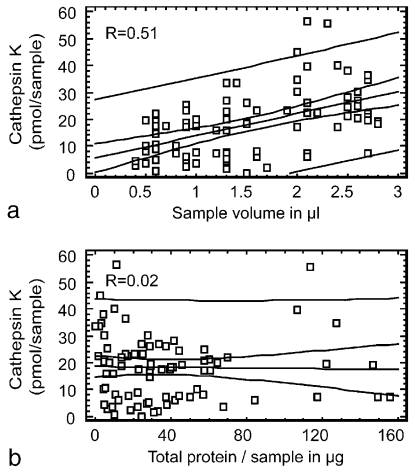

Fig. 3. Correlation of cathepsin K with volume of crevicular fluid and total protein. Absolute amount of cathepsin K per filter strip normalized to the time of collection *versus* the corresponding adsorbed volume of crevicular fluid. (a) and total protein of crevicular fluid (b) from peri-implantitis and control implants. *R* = correlation coefficient.

variable and MPI as independent variable, the correlation coefficient was 0.77. The correlation coefficients indicate a moderately strong relationship between the variables (Table 1).

Table 1. Correlation of clinical parameters: pocket probing depth (PD), modified bleeding index (MBI) and modified plaque index (MPI)

	MBI	MPI
PD	0.82	0.66
MBI		0.77

Data indicate the correlation coefficient *R*.

Sample homogeneity analysis: correlation with age; comparison between buccal and lingual sites, male and female, mandible and maxilla

Patients' age correlated with sample volume and with cathepsin K normalized to time, but not with cathepsin K normalized to adsorbed volume. Subanalysis of the two groups showed a correlation between age and adsorbed volume in the peri-implantitis group, whereas in the control group no significance was reached. No correlation was found when subanalysis of cathepsin K normalized by time and volume was performed (Table 2). Comparison of subgroups resulted in significant differences between male and female, and between mandible and maxilla, but not between buccal and lingual sites when analysed for the parameters adsorbed volume and cathepsin K normalized to time (Table 3B).

Discussion

Peri-implantitis is characterized by the accumulation of chronically activated inflammatory cells around implants, providing a microenvironment that stimulates osteoclast formation and activation (Gualini & Berglundh 2003, Bullon et al. 2004). Cathepsin K is a protease, which is highly expressed in osteoclasts and released as active enzyme into the pericellular space (Rantakokko et al. 1996, Dodds et al. 2001, Rieman et al. 2001). We therefore hypothesized that peri-implantitis is associated with increased levels of cathepsin K in the crevicular fluid. The data showed that the total amount of cathepsin K and the volume of crevicular fluid adsorbed to filter strips were both higher in the peri-implantitis group than in the group with healthy peri-implant tissue. Also, the total amount of gelatinase B, another protease highly expressed in osteoclasts, was increased in the crevicular fluid of patients with peri-implant bone loss

Table 2. Correlation of subjects' age with adsorbed volume, absolute amount of cathepsin K normalized by time and adsorbed volume

	Volume	Cathepsin K (pmol/sample)	Cathepsin K (nM)
Total	$R = 0.39$ ($p < 0.01$)	$R = 0.24$ ($p < 0.05$)	$R = -0.02$ ($p = 0.86$)
Control	$R = 0.29$ ($p = 0.07$)	$R = 0.22$ ($p = 0.18$)	$R = 0.08$ ($p = 0.64$)
Peri-implantitis	$R = 0.36$ ($p < 0.05$)	$R = 0.11$ ($p = 0.48$)	$R = -0.06$ ($p = 0.71$)

Table 3. Comparison of sample volume, absolute amount of cathepsin K normalized by time and to adsorbed volume between buccal and lingual sites, male and female, and mandible and maxilla

Number of samples	Total	Control	Peri-implantitis
(A)			
Buccal	40	20	20
Lingual	40	20	20
Male	24	4	20
Female	56	36	20
Mandible	54	24	30
Maxilla	26	16	10
	Volume (μl)	Cathepsin K pmol/sample	Cathepsin K nM
(B)			
Buccal	1.5 (0.4–2.7)	3.0 (0.0–9.0)	0.8 (0.0–2.4)
Lingual	1.3 (0.4–2.8)	2.9 (0.1–7.2)	0.8 (0.1–2.6)
Statistic	$p = 0.99$	$p = 0.93$	$p = 0.99$
Male	2.5 (0.7–2.8)	4.1 (2.8–9.0)	0.8 (0.4–1.7)
Female	1.2 (0.4–2.7)	1.6 (0.0–8.9)	0.7 (0.0–2.6)
Statistic	$p < 0.01$	$p < 0.01$	$p = 0.18$
Maxilla	1.3 (0.4–2.8)	1.3 (0.2–8.9)	0.5 (0.1–1.7)
Mandible	1.8 (0.5–2.8)	3.4 (0.0–9.0)	0.8 (0.0–2.6)
Statistic	$p < 0.05$	$p < 0.01$	$p = 0.08$

(Ma et al. 2003). Together, these findings suggest that proteolytic enzymes characteristically released by osteoclasts may be determinants of peri-implant tissue health.

The volume of adsorbed crevicular fluid increased with the clinical parameters of peri-implantitis. This common finding can be explained by changes in vascular permeability causing increased amounts of exudate in deep pockets (Griffiths et al. 1992, Kaklamanos & Tsalikis 2002, Griffiths 2003). Although the increased absolute amount of cathepsin K and the volume of crevicular fluid may be clinically relevant, the pathophysiologic background of the disease seems to be more complex, because the ratio between the two parameters is constant. When normalized to the adsorbed volume of crevicular fluid, cathepsin K levels even showed a moderately negative correlation with the clinical parameters of peri-implantitis. This slightly negative correlation might be because of active cathepsin K bound to the solid extracellular matrix substrates. When normalized to eluted protein, cathepsin K levels were not increased. A reliable biochemical parameter for monitoring peri-implant tissue health is expected to increase over-proportionally with the adsorbed volume of crevicular fluid. The data presented here show that evaluating cathepsin K with a combination of the intra-crevicular sampling method and immunoassay does not meet the requirements of a detection marker for peri-implantitis. As the total amount of cathepsin K correlates with the adsorbed volume obtained by intra-crevicular sampling, evaluating cathepsin K does not provide any information about the status of the disease other than that provided by volume measurements alone. However, samples were not equally distributed with regard to sex and collection site. Correlation analysis further indicates that sample volume may be dependent on the age of the subject. This should be considered in future studies to avoid interfering influences because of sample inhomogenity. In addition, the possible influence of the type of implant and prosthesis might be relevant for the outcome of future studies.

Peri-implant bone loss was reported to be 0.9–1.6 mm during the first year after implantation and 0.02–0.15 mm in the following years (Cox & Zarb 1987, Chaytor et al. 1991). Bone resorption during progressive peri-implantitis is not a linear process, as controlled animal studies show (Marinello et al. 1995, Tillmanns et al. 1997, Zitzmann et al. 2004). Consequently, biochemical parameters of bone resorption may reflect the active stage of tissue destruction and the silent phase of the disease. Therefore, prospective longitudinal studies are needed to correlate disease progression with biochemical parameters of bone resorption within the peri-implant crevicular fluid, such as cathepsin K. These studies could help to find an early diagnostic marker of peri-implantitis before clinical parameters are measurable. The data from such longitudinal studies could well contribute to pinpointing the transition from peri-implant mucositis to peri-implantitis. Moreover, they could yield information about the pathophysiological mechanisms underlying the different stages of peri-implantitis.

Acknowledgements

The authors wish to thank M. Pensch for her skilful technical assistance. The work was supported by a grant of the Austrian National Bank (Grant holder R. G.).

References

Ataoglu, H., Alptekin, N. O., Haliloglu, S., Gursel, M., Ataoglu, T., Serpek, B. & Durmus, E. (2002) Interleukin-1beta, tumor necrosis factor-alpha levels and neutrophil elastase activity in peri-implant crevicular fluid. *Clinical Oral Implants Research* **13**, 470–476.

Bossard, M. J., Tomaszek, T. A., Thompson, S. K., Amegadzie, B. Y., Hanning, C. R., Jones, C., Kurdyla, J. T., McNulty, D. E., Drake, F. H., Gowen, M. & Levy, M. A. (1996) Proteolytic activity of human osteoclast cathepsin K. Expression, purification, activation, and substrate identification. *Journal of Biological Chemistry* **271**, 12517–12524.

Boyle, W. J., Simonet, W. S. & Lacey, D. L. (2003) Osteoclast differentiation and activation. *Nature* **423**, 337–342.

Buchmann, R., Hasilik, A., Van Dyke, T. E. & Lange, D. E. (2002) Amplified crevicular leukocyte activity in aggressive periodontal disease. *Journal of Dental Research* **81**, 716–721.

Buhling, F., Waldburg, N., Gerber, A., Hackel, C., Kruger, S., Reinhold, D., Bromme, D., Weber, E., Ansorge, S. & Welte, T. (2000) Cathepsin K expression in human lung. *Advances in Experimental Medicine and Biology* **477**, 281–286.

Bullon, P., Fioroni, M., Goteri, G., Rubini, C. & Battino, M. (2004) Immunohistochemical analysis of soft tissues in implants with healthy and peri-implantitis condition, and aggressive periodontitis. *Clinical Oral Implants Research* **15**, 553–559.

Chambers, T. J. (2000) Regulation of the differentiation and function of osteoclasts. *Journal of Pathology* **192**, 4–13.

Chaytor, D. V., Zarb, G. A., Schmitt, A. & Lewis, D. W. (1991) The longitudinal effectiveness of osseointegrated dental implants. The Toronto study: bone level changes. *International Journal of Periodontics Restorative Dentistry* **11**, 112–125.

Chen, H. Y., Cox, S. W. & Eley, B. M. (1998) Cathepsin B, alpha2-macroglobulin and cystatin levels in gingival crevicular fluid from chronic periodontitis patients. *Journal of Clinical Periodontology* **25**, 34–41.

Cox, J. F. & Zarb, G. A. (1987) The longitudinal clinical efficacy of osseointegrated dental implants: a 3-year report. *International Journal of Oral Maxillofacial Implants* **2**, 91–100.

De Smet, E., van Steenberghe, D., Quirynen, M. & Naert, I. (2001) The influence of plaque and/or excessive loading on marginal soft and hard tissue reactions around Branemark implants: a review of literature and experience. *International Journal of Periodontics Restorative Dentistry* **21**, 381–393.

Dickinson, D. P. (2002) Cysteine peptidases of mammals: their biological roles and potential effects in the oral cavity and other tissues in health and disease. *Critical Reviews in Oral Biology and Medicine* **13**, 238–275.

Dodds, R. A., James, I. E., Rieman, D., Ahern, R., Hwang, S. M., Connor, J. R., Thompson, S. D., Veber, D. F., Drake, F. H., Holmes, S., Lark, M. W. & Gowen, M. (2001) Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption. *Journal of Bone and Mineral Research* **16**, 478–486.

Esposito, M., Hirsch, J. M., Lekholm, U. & Thomsen, P. (1998) Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. *European Journal of Oral Science* **106**, 527–551.

Gowen, M., Lazner, F., Dodds, R., Kapadia, R., Feild, J., Tavaria, M., Bertoncello, I., Drake, F., Zavarselk, S., Tellis, I., Hertzog, P., Debouck, C. & Kola, I. (1999) Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. *Journal of Bone and Mineral Research* **14**, 1654–1663.

Griffiths, G. S. (2003) Formation, collection and significance of gingival crevice fluid. *Periodontology 2000* **31**, 32–42.

Griffiths, G. S., Sterne, J. A., Wilton, J. M., Eaton, K. A. & Johnson, N. W. (1992) Associations between volume and flow rate of gingival crevicular fluid and clinical assessments of gingival inflammation in a population of British male adolescents. *Journal of Clinical Periodontology* **19**, 464–470.

Gualini, F. & Berglundh, T. (2003) Immunohistochemical characteristics of inflammatory lesions at implants. *Journal of Clinical Periodontology* **30**, 14–18.

Ida-Yonemochi, H., Ishibashi, O., Sakai, H. & Saku, T. (2004) Recruitment of osteoclasts in the mandible of osteopetrotic (op/op) mice. *European Journal of Oral Science* **112**, 148–155.

Kaklamanos, E. G. & Tsalikis, L. (2002) A review on peri-implant crevicular fluid assays potential in monitoring and predicting peri-implant tissue responses. *Journal of International Academy Periodontology* **4**, 49–59.

Kivela-Rajamaki, M., Maisi, P., Srinivas, R., Tervahartiala, T., Teronen, O., Husa, V., Salo, T. & Sorsa, T. (2003a) Levels and molecular forms of MMP-7 (matrilysin-1) and MMP-8 (collagenase-2) in diseased human peri-implant sulcular fluid. *Journal of Periodontal Research* **38**, 583–590.

Kivela-Rajamaki, M. J., Teronen, O. P., Maisi, P., Husa, V., Tervahartiala, T. I., Pirila, E. M., Salo, T. A., Mellanen, L. & Sorsa, T. A. (2003b) Laminin-5 gamma2-chain and collagenase-2 (MMP-8) in human peri-implant sulcular fluid. *Clinical Oral Implants Research* **14**, 158–165.

Kiviranta, R., Morko, J., Uusitalo, H., Aro, H. T., Vuorio, E. & Rantakokko, J. (2001) Accelerated turnover of metaphyseal trabecular bone in mice overexpressing cathepsin K. *Journal of Bone and Mineral Research* **16**, 1444–1452.

Kourtis, S. G., Sotiriadou, S., Voliotis, S. & Challas, A. (2004) Private practice results of dental implants. Part I: survival and evaluation of risk factors Part II: surgical and prosthetic complications. *Implant Dentistry* **13**, 373–385.

Lam, J., Takeshita, S., Barker, J. E., Kanagawa, O., Ross, F. P. & Teitelbaum, S. L. (2000) TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *Journal of Clinical Investigation* **106**, 1481–1488.

Lindeman, J. H., Hanemaaijer, R., Mulder, A., Dijkstra, P. D., Szuhai, K., Bromme, D., Verheijen, J. H. & Hogendoorn, P. C. (2004) Cathepsin K is the principal protease in giant cell tumor of bone. *American Journal of Pathology* **165**, 593–600.

Liskmann, S., Zilmer, M., Vihalemm, T., Salum, O. & Fischer, K. (2004) Correlation of peri-implant health and myeloperoxidase levels: a cross-sectional clinical study. *Clinical Oral Implants Research* **15**, 546–552.

Littlewood-Evans, A. J., Bilbe, G., Bowler, W. B., Farley, D., Wlodarski, B., Kokubo, T., Inaoka, T., Sloane, J., Evans, D. B. & Gallagher, J. A. (1997) The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Research* **57**, 5386–5390.

Ma, J., Kitti, U., Hanemaaijer, R., Teronen, O. P., Sorsa, T. A., Natah, S., Tensing, E. K. & Konttinen, Y. T. (2003) Gelatinase B is associated with peri-implant bone loss. *Clinical Oral Implants Research* **14**, 709–713.

Marinello, C. P., Berglundh, T., Ericsson, I., Klinge, B., Glantz, P. O. & Lindhe, J. (1995) Resolution of ligature-induced peri-implantitis lesions in the dog. *Journal of Clinical Periodontology* **22**, 475–479.

Mombelli, A. & Lang, N. P. (1994) Clinical parameters for the evaluation of dental implants. *Periodontology 2000* **4**, 81–86.

Mombelli, A., van Oosten, M. A., Schurch, E. Jr & Land, N. P. (1987) The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiology and Immunology* **2**, 145–151.

Murata, M., Tatsumi, J., Kato, Y., Suda, S., Nunokawa, Y., Kobayashi, Y., Takeda, H., Araki, H., Shin, K., Okuda, K., Miyata, T. & Yoshie, H. (2002) Osteocalcin, deoxypyridinoline and interleukin-1beta in peri-implant crevicular fluid of patients with peri-implantitis. *Clinical Oral Implants Research* **13**, 637–643.

Nishi, Y., Atley, L., Eyre, D. E., Edelson, J. G., Superti-Furga, A., Yasuda, T., Desnick, R. J. & Gelb, B. D. (1999) Determination of bone markers in pycnodysostosis: effects of cathepsin K deficiency on bone matrix degradation. *Journal of Bone and Mineral Research* **14**, 1902–1908.

Oringer, R. J., Palys, M. D., Iranmanesh, A., Fiorellini, J. P., Haffajee, A. D., Socransky, S. S. & Giannobile, W. V. (1998) C-telopeptide pyridinoline cross-links (ICTP) and periodontal pathogens associated with endosseous oral implants. *Clinical Oral Implants Research* **9**, 365–373.

Pjetursson, B. E., Tan, K., Lang, N. P., Bragger, U., Egger, M. & Zwahlen, M. (2004) A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. *Clinical Oral Implants Research* **15**, 625–642.

Rantakokko, J., Aro, H. T., Savontaus, M. & Vuorio, E. (1996) Mouse cathepsin K: cDNA cloning and predominant expression of the gene in osteoclasts, and in some hypertrophying chondrocytes during mouse development. *FEBS Letters* **393**, 307–313.

Rieman, D. J., McClung, H. A., Dodds, R. A., Hwang, S. M., Holmes, M. W., James, I. E., Drake, F. H. & Gowen, M. (2001) Biosynthesis and processing of cathepsin K in cultured human osteoclasts. *Bone* **28**, 282–289.

Saftig, P., Hunziker, E., Wehmeyer, O., Jones, S., Boyde, A., Rommerskirch, W., Moritz, J. D., Schu, P. & von Figura, K. (1998) Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. *Proceedings of the National Academy of Science USA* **95**, 13453–13458.

Salvi, G. E. & Lang, N. P. (2004) Diagnostic parameters for monitoring peri-implant conditions. *International Journal of Oral Maxillofacial Implants* **19** (Suppl.), 116–127.

Skoumal, M., Haberhauer, G., Kolarz, G., Hawa, G., Woloszczuk, W. & Klingler, A. (2005) Serum cathepsin K levels of patients with longstanding rheumatoid arthritis: correlation with radiological destruction. *Arthritis Research Therapy* **7**, R65–R70.

Soell, M., Elkaim, R. & Tenenbaum, H. (2002) Cathepsin C, matrix metalloproteinases, and their tissue inhibitors in gingiva and gingival

crevicular fluid from periodontitis-affected patients. *Journal of Dental Research* **81**, 174–178.

Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M. T. & Martin, T. J. (1999) Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocrine Reviews* **20**, 345–357.

Teitelbaum, S. L. (2000) Bone resorption by osteoclasts. *Science* **289**, 1504–1508.

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

Tervahartiala, T., Konttinen, Y. T., Ingman, T., Hayrinen-Immonen, R., Ding, Y. & Sorsa, T. (1996) Cathepsin G in gingival tissue and crevicular fluid in adult periodontitis. *Journal of Clinical Periodontology* **23**, 68–75.

Tillmanns, H. W., Hermann, J. S., Cagna, D. R., Burgess, A. V. & Meffert, R. M. (1997) Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part I. Clinical evaluation. *International Journal of Oral Maxillofacial Implants* **12**, 611–620.

Tonetti, M. S. & Schmid, J. (1994) Pathogenesis of implant failures. *Periodontology 2000* **4**, 127–138.

Trabandt, A., Muller-Ladner, U., Kriegsmann, J., Gay, R. E. & Gay, S. (1995) Expression of proteolytic cathepsins B, D, and L in periodontal gingival fibroblasts and tissues. *Laboratory Investigation* **73**, 205–212.

Tsuji, Y., Yamaza, T., Kido, M. A., Goto, T., Nakata, S., Akamine, A., Nakasima, A. & Tanaka, T. (2001) Expression of cathepsin K mRNA and protein in odontoclasts after experimental tooth movement in the mouse maxilla by in situ hybridization and immunoelectron microscopy. *Cell Tissue Research* **303**, 359–369.

Wang, D., Li, W., Pechar, M., Kopeckova, P., Bromme, D. & Kopecek, J. (2004) Cathepsin K inhibitor-polymer conjugates: potential drugs for the treatment of osteoporosis and rheumatoid arthritis. *International Journal of Pharmaceutics* **277**, 73–79.

Wei, S., Kitaura, H., Zhou, P., Ross, F. P. & Teitelbaum, S. L. (2005) IL-1 mediates TNF-induced osteoclastogenesis. *Journal of Clinical Investigation* **115**, 282–290.

Yamashita, D. S. & Dodds, R. A. (2000) Cathepsin K and the design of inhibitors of cathepsin K. *Current Pharmaceutical Design* **6**, 1–24.

Zitzmann, N. U., Berglundh, T., Ericsson, I. & Lindhe, J. (2004) Spontaneous progression of experimentally induced periimplantitis. *Journal of Clinical Periodontology* **31**, 845–849.

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Clinical Relevance

Scientific rationale for the study: Cathepsin K is characteristically expressed by osteoclasts in high concentrations and was therefore proposed as a predictive marker of peri-implantitis.

Principal findings: We found a positive correlation between the total amount of cathepsin K with the clinical parameters of peri-implantitis and the volume adsorbed to the filter strips after standardized intra-crevicular fluid sampling.

Practical implications: According to the data obtained in this pilot study, evaluating cathepsin K with a combination of intra-crevicular sampling and immunoassay does not yet meet the requirements of a diagnostic marker for peri-implantitis.