# Bone Loss Biomarkers Evaluation as an Early Diagnosis and Prognostic Factor of Peri-implantitis

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*Peri-implantitis presents inflammatory process that affects soft and hard supporting tissues of osseointegrated implant. Inflammatory processes that occur in the case of periodontitis and in the case of peri-implantitis have similar characteristics, with bone loss as defining element. The studies have shown that peri-implant and gingival sulcus have a similar structure, sharing the same immune components in the inflammatory process. At molecular level, the balance between bone-resorbing osteoclasts and bone-producing osteoblasts is mediated by RANK (receptor activator of nuclear factor kappa B). The current scientific data doesn't offer specific information regarding the role of RANK in the process of bone resorption in peri-impantitis/periodontitis. The aim of this study was to investigate if there are any correlations between receptor activator of nuclear factor kappa B (RANK) concentrations in peri-implant crevicular fluid and clinical parameters that reflect inflammatory nature of peri-implantitis.*

*Keywords: RANK, peri-implantitis, dental implant, periodontitis*

The inflammatory process of surrounding tissue will lead to bone lost around dental implant [1]. On molecular scale, the balance between bone-resorbing osteoclasts and bone-producing osteoblasts is mediated by receptor activator of nuclear factor kappa B (RANK), together with a receptor-like molecule named osteoprotegerin (OPG) and his ligand (RANKL) [2]. These receptors are members of tumor necrosis factor (TNF) superfamily, which play a crucial role in the bone remodelling process [2,3]. RANK is localized on the surfaces of preosteoclasts and osteoclasts. The ligation with a specific ligand, RANKL, leads to differentiation and maturation of progenitor cells simultaneously with osteoclasts activity enhancement which leads to high level of pro-inflammatory cytokines regulated by RANK [4]. In this context, OPG appear as a receptor-like glycoprotein synthetize in human cells playing a regulatory role in the OPG/RANK/RANKL chain [5]. OPG can inhibit RANK-RANKL interaction followed by a decrease in osteoclasts activity and also reducing their number [6]. RANKL could be found in soluble form or expressed by osteoblasts, stromal cells, fibroblasts, B-cells and T-cells and in different hormonal stimulation [7]. Thus, given the structure more than similar between marginal periodontium tissues and peri-implantar fluids expressed at this level can be considered to be analytical and diagnostic markers involved in bone metabolism. Recent studies demonstrating a reflection at this level of the surrounding tissue, trough the volume and composition of these fluids, depending directly on the health of the surrounding tissue. The scientific literature presents several lines of research on the OPG / RANK / RANKL system and its participation in the metabolism of bone around teeth and dental implants. However there are no clear results regarding the role of RANK associated with clinical parameters that reflect inflammatory nature of peri-implantitis and periodontitis.

## Experimental part

### *Materials and method*

The study was conducted between 2014-2015 in a private dental practice. The study was conducted according to the Helsinki Declaration of 1975, revised in 2000. The study protocol was approved by the Ethics Committee of the Faculty of Dentistry, University of Medicine and Pharmacy *Gr.T.Popa* Iasi and every person involved in study signed informed consent. The study included 62 patients divided into 3 groups: healthy implants (n = 21) periodontitis (n = 21) and peri-implantitis (n = 20). Patients included in the study were selected and divided into groups according to the criteria for inclusion. Patients with healthy implants: without any clinical signs of inflammation, BOP = 0 and PPD ≤ 3mm, absence of radiological bone resorption, no gingival retraction, at least 1 year old prosthetics restoration. Periodontitis patients: deep marginal periodontitis present, according to Carranza classification, involving at least 3 teeth, periodontal pocket depth (PPD) ≥ 5 mm, radiological signs of bone resorption. Peri-implantitis: at least one implant with peri-implantitis, at least 1 year old prosthetics restoration, peri-implant pocket depth (PPD) ≥ 5 mm, gingival recession relative clinical attachment level (rCAL ) ≥ 4 mm, positive bleeding on probing (BOP) and radiographic bone loss involving ≥ 2 threads compared to radiography taken at the time of prosthetic placement. Patients recruited in the three groups are healthy systemically, non-smokers and not place themselves in one of the following conditions: treatment for periodontitis or peri-implantitis in the last year, usage of antibiotics and anti-inflammatory agents within the preceding 3 months, menstruation, pregnancy and lactation in female patients.

### *Clinical examination*

This was the cross-sectional study which will compare clinical and biochemical parameters of the three groups. A complete periodontal and peri-implantar evaluation were conduct on every patient, and were measured the following parameters for each tooth taken in study: PPD (mm), BOP - presence (1) or absence (0) of bleeding for up to 15 s after probing, visible plaque accumulation (Pl): presence (1) or absence (0) of plaque along the mucosal margin. The clinical parameters were measured in 6 points: mesio-bucal, medio-bucal, disto-bucal, mesio-lingual, medio-lingual and disto-lingual. For each tooth / implant was chosen as representative site the one with maximum

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depth of the pockets, and for equal values, was chosen the bucal site due to easier access to the clinical examination. All measurements were performed by the one same trained and calibrated examiner using the same type of the graduated periodontal probe. Intra-examiner calibration was performed twice, before and during the study. Intraoral radiographies were performed for radiological evidence of bone loss using paralleling technique.

*Biochemical sample collection*

Test teeth were isolated with cotton rolls and dry air jet. The peri-implant crevicular fluid (PICF) and gingival collection was performed using sterile paper cones, with a standard dimension no. 40 (Whatman no. 1), inserted into the selected sites, gently, until mild resistance, keeping the cone in place for 30 s. Cones visibly contaminated with blood or saliva were not taken into account. Once they have been removed, the cones were placed in special tubes (with 100 µL of sterile phosphate buffered saline) and weighed, to measure the volume of the peri-implant crevicular fluid (PICF) and gingival crevicular fluid (GCF). Before being frozen for storage until processing, was determine the volume of PICF / GCF by weighing cones compared to baseline dry cones, avoiding as much as possible evaporation that would change the values actual volume. The sampling time method which includes a total amount of RANK in picograms (pg) per site during 30 s was chosen because the method was supported by previous studies as convenient for related researches [8]. The samples were stored at -70°C until enzyme-linked immune-sorbent assay (ELISA) analysis

*Determination of RANK using ELISA*

To determine the concentration of RANK in PICF and GCF was used a commercial ELISA Kit Kit (RayBio® Sandwich ELISA kits) according to the manufacturer's protocol and recommendations. Determination of RANK was obtained by spectrophotometric assessment (Star Fax 4200 analyser). The concentration result was obtained in picomoles of RANK. After conversion, the final value pg / mL of RANK per sample is obtained.

*Statistical analysis*

The primary variable is the concentration of RANK for each sample. Secondary variables are the bleeding index, plaque index, periodontal pocket depth, PICF volume, GCF volume. Statistical analysis was performed using SPSS for Windows.

## Results and discussions

The study included 62 patients, 29 patients were men and 33 women, aged between 26-61 years. The volume of collected PICF/GCF was similar in samples of all the investigated groups. All clinical parameters analyzed were significantly higher in the group with periodontal and peri-implantation group compared with healthy patients (control group, P <0.000). As shown in table 1, there are significant differences between the mean values obtained for the 3 clinical parameters (PD / PDD, GFC / PICF and RANK) in patients group with periodontitis and peri-implantitis. All GFC / PICF samples assessed were included in the study, the values were above the detection limit of the method.

RANK concentration was significantly higher in the group with periodontitis compared with healthy group (P = 0.000) also in the group with peri-implantitis compared to healthy patient group (P = 0.000). Thus, RANK concentration is significantly higher in patients with peri - implantitis ($M_{Peri\text{-}implantitis}$ = 361.1845 pg/mL) in comparison with control group ($M_{Control}$ = 244.6762 pg/mL), with a statistical difference between the two values ($P$<0.001) (fig. 1).

| | Control | Periodontitis | Peri-implantitis |
|---|---|---|---|
| PD/PPD (mm) | 2.10 ± 0.70 | 5.5 ± 0.74 | 5.00 ± 0.725 |
| PICF/GCF (mL) | 0.3810 ± 0.12720 | 0.5771 ± 0.12642 | 0.3735 ± 0.15935 |
| RANK (pg/mL) | 244.6762 ± 115.86265 | 828.5195 ± 100.41388 | 361.1845 ± 124.29966 |

PPD/PD –peri-implant pocket depth/pocket depth

PICF/GCF – peri-implant crevicular fluid/gingival crevicular fluid

RANK – receptor activator of nuclear factor kappa-B

**Table 1**
DESCRIPTIVE STATISTICS OF RANK CONCENTRATION AND THE MEASURED CLINICAL PARAMETERS AMONG THE GROUPS

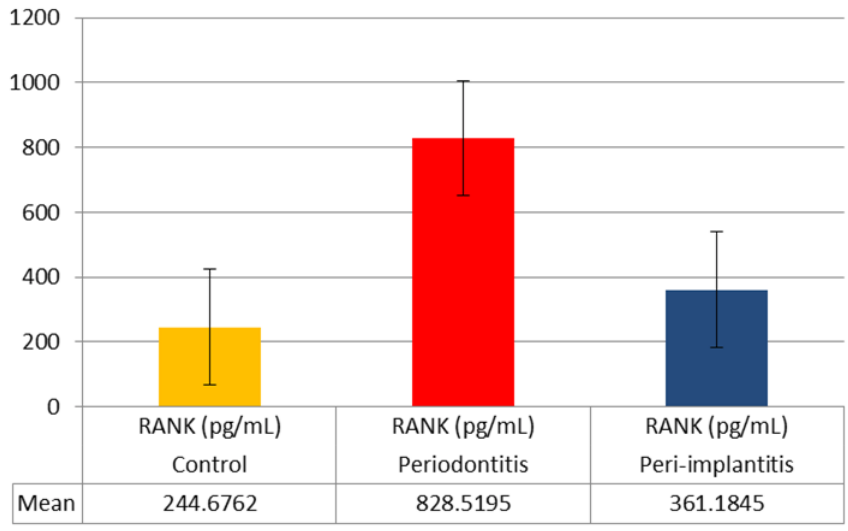


Fig. 1. Comparison of mean values of RANK between the groups

A variety of studies were dedicated to resolution of the multifactorial pathogenesis of peri-implantitis, aiming to increase the success rate of peri-implantar treatment success rate. However, a marker with predictive value of peri-implantar bone damage degree has not yet been identified in order to indicate an early diagnosis of this condition. The role of the study is to verify the possibility of using RANK determination as a method for early diagnosis and prognosis of peri-implantitis.

The results obtained in the periodontitis group are related to those in the literature regarding the evaluation of biomarkers involved in osteoclast differentiation and bone resorption [9-11]. There are also studies that indicate RANK determination rather as an indicator of the degree of periodontal [12, 13]. Although peri-implantitis and periodontitis have a similar pathogenesis, mainly characterized by a chronic inflammatory process as a result of biofilm bacterial aggression, the two conditions have aspects that differentiate them, mainly characterized by a directly propagation of inflammation to the bone in the case of peri-implantitis [14].

The results of this study show a RANK increase in patients with peri-implantitis group versus healthy implants group. Furthermore, a significant difference was observed between the values of RANK, PPD and PICF and the presence of other clinical indicators, as plaque index and BOP in the peri-implantitis group compared to healthy implant group. In the group of patients with periodontal disease there were also obtained high values of these parameters as compared to healthy patients group. In contrast, the values obtained in the group of patients with periodontitis were significantly higher compared to patients with peri-implantitis [15].

Due to lack of data in the literature on this biomarker analysis it is difficult to compare results. In a study by Rakic M. et al. 2012 [14] assessing RANK concentration from GCF / PCIF of 70 patients there was found average values in the intervals obtained in this study, but in this case, the average RANK values was higher in the group with peri-implantitis compared to periodontitis group. However, the results of Rakic M. et al. indicate a significant growth of the biomarker in lots of conditions present to the group with healthy patients. Another study by Sarlati F. et al. [16] in 2010 on 40 implants divided into three groups (clinically healthy, peri-implantar mucositis and peri-implantitis) assessing the average amount of RANK-L led to the conclusion that there is no statistics differences between the groups, regardless of the amount of GCF / PICF obtained or how the was the PD / PPD. These results suggest that RANK is the only biomarker that varies depending on the degree of bone damage, being dependent of the amount of GCF / PICF and PD / PPD value.

Rakic M. et al. in a study conducted in 2013 [11] on 67 patients, obtained average values in the same range as those determined. RANK values of periodontitis and peri-implantitis groups are also several times higher than those obtained in the group of healthy patients, concluding that there is a direct relationship between the depth of the pockets, the amount of crevicular fluid and the RANK value and also, the degree of periodontal and peri-implantar damage.

Another mechanism of activation of the receptor is self-ligation by increasing the concentration of pro-inflammatory cytokines derived from the bacterial load [17]. This way, can be explain the high level of RANK found in the peri-implant implants compared with healthy ones. Nowzari et al, in a study conducted in 2010 [18] showed high levels of pro-inflammatory cytokines in the healthy implants compared to periodontal tissue healthy, which can be explained by the existence of a mechanism for regulating the quantity of RANK and its activation, indicating an increase in osteoclast genesis, which is reported in peri-implantitis [19].

## Conclusions

The information presented is new, this paper demonstrates the direct dependence between the values of RANK and other biological parameters evaluated and their positive association with the presence of peri-implantar damage, and the association between these parameters and the periodontal and peri-implantar inflammatory osteo-destructive process.

The concentration of RANK can be used as a parameter that can be monitored in the diagnosis and prognosis of peri-implantitis. Also, this determination can be an easy method of tracking the success of treatment, and a target of future research regarding the mechanisms that occur in the peri-implantar bone destruction.

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