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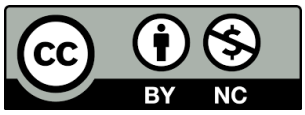

Is the Personalized Approach the Key to Improve Clinical Diagnosis of Peri-Implant Conditions? The Role of Bone Markers

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Summary sentence: Personalized model demonstrated capacity of BTMs to substantially improve capacity of clinical diagnosis, however the algorithms identified PIM cluster of patients exhibiting exceeding levels of bone loss markers thus suggesting the need for more refined definition of peri-implant conditions according to biological characteristics.

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Mia Rakic: clinical assessment, interpretation, paper drafting, approved final version

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ABSTRACT

Background: Study objectives were 1) to estimate diagnostic capacity of clinical parameters, receptor-activator-nuclear factor kappa-B (RANKL) and osteoprotegerin (OPG) to diagnose healthy peri-implant condition (HI), peri-implant mucositis (PIM) and peri-implantitis (PIMP) by assessing respective diagnostic accuracy, sensitivity, specificity and diagnostic ranges 2) to develop personalized diagnostic model (PDM) for implant monitoring.

Methods: Split-mouth study included 126 patients and 252 implants (HI=126, PIM=57 and PI=69). RANKL and OPG concentrations were estimated in peri-implant crevicular fluid using ELISA method and assessed with clinical parameters using routine statistics, while the diagnostic capacity of individual parameters and overall clinical diagnosis were estimated using classifying algorithms. PDM was developed using decision trees.

Results: Bleeding on probing (BOP), plaque index and probing depth (PD) were confirmed reliable discriminants between peri-implant health and disease, while increase in PD (PD>4mm) and SUP were good discriminants amongst PIM/ PIMP. BTMs demonstrated presence of bone resorption in PIM, comparable diagnostic ranges between PIM/PIMP, PIMP was clinically distinguished from PIM

in about 60% of patients while 40% remined diagnosed as false negatives. PDM demonstrated highest diagnostic capacity (accuracy: 96.27%, sensitivity: 95.00%, specificity: 100%) and defined HI: BOP≤0.25%; PIM: BOP>0.25%, PD≤4.5mm; PIMP: BOP>0.25%, PD>4.5mm and RANKL≤19.9 pg/site; PIM: BOP>0.25%, PD>4.5mm and RANKL>19.9 pg/site.

Conclusion: BTMs demonstrated capacity to substantially improve clinical diagnosis of peri-implant conditions. Considering lack of difference in BTMs between PIM/ PIMP and cluster of PIM with exceeding BTMs, a more refined definition of peri-implant conditions according to biological characteristics is required for further BTMs validation and appropriate PIMP management.

Keywords: Peri-implantitis; peri-implant mucositis, diagnosis; biomarkers; Precision medicine; Personalized Medicine

INTRODUCTION

Peri-implantitis is a leading cause of implant failure [1,2] that affects roughly 2 out of 10 patients This inflammatory condition is characterized by asymptomatic and fast progressing bone resorption, while the respective predictive treatment protocol is still not established [4–6]. Considering estimated rate of 5 million implants placed annually, peri-implantitis (PIMP) represents a growing problem in dentistry [7] with negative impact in cost-effectiveness of the leading treatment protocol in restorative dentistry [8]. In context of such alerting facts, early diagnosis of pathological bone resorption remains of crucial importance for PIMP management and emerging solution until development of more predictive treatment protocols. In brief, the extension of soft tissue inflammation to the bone turns disease course into an irreversible, non-linear fast progressing pattern[6]. This is followed by formation of crater-like

defects and wide contamination of implant surface currently being considered the major cause of unsuccessful treatment [5,6]. Moreover, the research studies unceasingly reveals a magnitude of interfering factors that additionally complicates PIMP diagnosis and related managment[9]. In that context, early detection of PIMP prevents the need for advanced treatment approach, thus contributing to the more predictive treatment success and better cost-effectiveness.

Clinical diagnosis of peri-implant conditions is built on combined and comparative assessment of bone changes and clinical parameters [10] since structural specificities of peri-implant tissues, more expressed bone remodeling, variation in implant designs and implantation techniques substantially compromise standardization of crucial clinical parameters on implants [11–14]. Hence, in lack of periodontal fibers the periodontal probe penetrates apically from junctional epithelium that is additionally facilitated under inflammation[15], while the peri-implant tissues seems to be more prone on bleeding on probing.

Therefore, even the recent classification relies on a comparative approach both for probing depth (PPD) and for radiological bone loss (RXBL), while in lack of serial clinical records, the thresholds are radically increased on PPD>6mm and RXBL>3mm to ensure the diagnosis [16]. Subsequently, a decreased diagnostic sensitivity of clinical parameters [17] is followed by increased rate of false negative cases that particular impacts diagnosis of peri-implantitis onset and early bone lesions[18]. The multifactorial pathology with disease complexity that exceeds capacity of clinical diagnosis, with increasing prevalence and negative impact in healthcare cost represents a major indication for personalized approach.[19,20] Personalized medicine implements clinical and biological parameters into highly precise diagnostic/treatment strategy tailored to the individual case thus indeed overcoming limitations of the binary 20th century “one size fits all” medical approach. Hence, the biomarkers were introduced for the first time in the new classification of periodontal and implant conditions as an onward or step towards implementation of precision medicine in clinical practice.[21] On this occasion, the expert team emphasized the importance of comprehensive diagnosis of

periodontal/peri-implant conditions according to biological characteristics and expressed the lack of diagnostic biomarkers by interrogation marks in the main classification schema.

Bone turnover markers (BTM) comprise the fast-responsive regulators and byproducts of the bone metabolism able to provide objectively measurable diagnostic information on ongoing bone processes [22]. Hence, the clinical utility of BTM remains outstanding since the highly sensitive diagnostic assays are able to capture their respective shift in concentrations before clinically manifested changes thus overcoming the mm-level clinical measurements. Currently, there is no biomarker standardized for diagnostic use in implantology since reported biomarker studies were mostly oriented towards inflammatory profiling and did not focus on biomarker validation [18]. This research team worked on validation of biomarkers for diagnosis of peri-implant conditions according to comprehensive recommendations. [23–25] Thus the pre-analytical and analytical protocols were optimized for biomarker measurement in PICF specimens, while the receptor activator nuclear-kB (RANK), RANK-ligand (RANKL), osteoprotegerin (OPG), cathepsin-K and sclerostin were estimated as candidate markers [26,27]. Based on the high detectability and reproducibility rate and high-rate correlation with standard clinical endpoints[23–25], RANKL and OPG were identified as the most suitable biomarker candidates for peri-implant monitoring. Such promising diagnostic capacity of RANKL and OPG ensues from the fact that these mediators represent the key regulators of inflammatory osteoclastogenesis representing a pathognomonic process in PIMP.[28] Therefore, the changes in RANKL and OPG levels are able to reflect the onset and activity of bone resorption in the real-time.

This research team recently assessed diagnostic capacity of clinical implant diagnosis and confirmed that the individual clinical parameters are unable to provide accurate implant diagnosis, while assessment of the panel of clinical parameters improved respective accuracy confirming the limitations of clinical parameters [17]. In the spirit of demonstrated limitations, within implications for future research it has been proposed the use of personalized approach combining clinical and

biological markers as promising solution to improve implant diagnostics. Currently, there is a strong tendency in medicine toward synthesis of data-driven and expert-knowledge into personalized approach, with a main objective of exploiting the outstanding statistical power of algorithms while guiding the process based on the clinical knowledge [29]. In brief, personalized medicine relies on sophisticated mathematical algorithms that cross-analyze a panel of clinical and biological markers and implement the "knowledge" into highly accurate clinical plan fitting the individual patient.

We hypothesized that biomarker-supported clinical diagnosis might provide more accurate diagnostic information on peri-implant condition thus compensating limitations of standard clinical parameters.

The objectives of the present study were: 1) to estimate diagnostic capacity of the standard clinical parameters and 2 bone turnover markers to diagnose healthy peri-implant condition, peri-implant mucositis and peri-implantitis by assessing respective diagnostic accuracy, sensitivity, specificity and diagnostic ranges 2) to develop personalized diagnostic model for implant monitoring based on the clinical parameters and bone markers.

MATERIAL AND METHODS

The present study was designed to estimate diagnostic capacity of the standard clinical parameters and BTMs for diagnosis of peri-implant conditions according to referent case definitions [10,16]. Since this was the first study to define diagnostic ranges of BTMs, the study was conducted in the split mouth design to limit the impact of inter-individual variability. Finally, the advanced algorithm was used to identify the critical diagnostic parameters for each peri-implant condition and to implement them into personalized diagnostic model (PDM) for clinical-decision making, that was additionally validated.

Study population

Study population comprised of patients attending the Clinic for Maxillofacial, Oral Surgery and Implantology, Military Medical Academy, Belgrade, Serbia from September 2010 until October 2017. The study was approved by the institutional ethics committee of the Medical Military Academy, Belgrade, Serbia, (permission reference # VMA10-12-A.1) and was conducted in accordance with the Helsinki declaration of 1975, as revised in 2013. All patients provided written informed consent for treatment.

Systemically healthy non-smokers having at least one implant with healthy peri-implant tissues and one implant with signs of inflammation loaded for >1 year were recruited as eligible and clustered according to the case definition proposed by Sanz & Chapple [10] and conformant to the recent classification of peri-implant conditions [16,30]:

1. Peri-implant mucositis (PIM)- implants with bleeding on probing (BOP)>0.16 (positive in>1 point), probing depth (PD)>3mm and radiological bone loss (RXBL) <2mm
2. Peri-implantitis (PIMP)- PD ≥5 mm, BOP>1 and RXBL involving ≥2mm compared to the radiograph taken at the time of prosthetic loading.

Healthy controls (HI) represented the implants with negative BOP or BOP positive in 1/6 sites being considered the consequence of trauma, with PD<3mm and without evidence of RXBL.

Exclusion criteria were the following: 1) Periodontal treatment in the preceding year; 2) Intake of antibiotics in the preceding 6 months and; 3) grade 4 periodontitis [31] and 4) pregnant or lactating females. In order to avoid the impact of iatrogenic factors, implant supported restoration showing the signs of biomechanical overload and cement remnants were excluded.

Clinical Outcome Variables

The full-mouth periodontal measurements were performed in six points per tooth using a periodontal probe graded in mm* to assess inter-group homogeneity regarding periodontal status:

- Full-mouth Bleeding on Probing (FMBOP)
- Full-mouth Plaque Index (FMPI)
- Full-mouth probing depth (PD)
- Full-mouth clinical attachment level (CAL)

The clinical examination of all implants present in the mouth was performed using a plastic probe graded in mm† to select the representative implant site. In case of more than one implant with the same condition, the implant with the worst clinical characteristics in case of inflammation conditions and the most accessible implant in case of healthy peri-implant tissues was selected as representative. The site related clinical measurements were recorded in 6 sites per implant applying 0.15N/cm force for each representative implant including: BOP, suppuration (SUP), PI and PD. The RXBL and respective change from the moment of prosthetic loading were linearly measured on the radiographs acquisitioned using a paralleling technique combined with long cone [32] being the implant shoulder the referent point. All measurements were performed by two experienced examiners (M.R. and Z.T.) after a calibration exercise demonstrating 95.7% concordance within ± 1 mm for measurements of PD.

Biochemical Outcome Measurements

Diagnostic specimens were retrieved at the baseline and further processed according to the previously optimized protocol for RANKL and OPG evaluation in PICF samples [26,27]. In brief, PICF specimens were collected 24-72h post-examination by placing the paper strips till mild resistance for 30s at the

mesial aspect of representative implants. The samples were stored in microcentrifuge plastic tubes containing 0.5 mL sterile phosphate-buffered saline and transferred to the laboratory for further analyses. The minimal detection limits of commercial ELISA assays[‡] were: sRANKL (0.2 pg/mL) and OPG (1.4 pg/mL). The biomarker concentrations were expressed as total biomarker amount (pg) per site in 30 seconds according to the sampling time method [33].

Data analysis

The primary outcome variables were PICF concentrations of sRANKL, OPG, RANKL/OPG as well as implant-site values of BOP, PI, PD and RXBL. In lack of referent diagnostic values for biomarkers, the sample size calculation was performed using RANKL values from the previous studies [26,27], thus a sample size of 36 participants using α of 0.05 would result in a power of 0.95. However, the sample was preventively increased considering undefined diagnostic ranges of the markers.

Full-mouth PI, BOP, CAL and PD were averaged by each present tooth and then calculated by patient and by group being the patient the unit of analysis. Age and clinical parameters were expressed by mean and standard deviations (SD) while distribution of periodontal status was expressed in percent. The normality was tested using Shapiro-Wilk test. Age, gender, FMBOP and FMPI were compared using Fisher's exact test, while the FMPD and FMCAL were compared using independent Student T-test. For the implant-site measurements the mean values were calculated for BOP, PI and PD per implant and then by group. The clinical parameters, RXBL and BTMs were compared between PIM, PIMP and Hi using Kruskal-Wallis test, the differences were evaluated using the Mann–Whitney test while the p-values were adjusted using Dunn's post hoc test hence the $p<0.016$ was considered as statistically significant. The correlations between BTMs and clinical parameters were assessed with the Spearman's rank correlation test. The statistical analysis was performed using commercial software[§].

Estimation of the diagnostic capacity of diagnostic parameters and development of Predictive Diagnostic Model (PDM)

The independent clinical parameters and BTMs were evaluated using classification models in order to estimate respective accuracy, specificity, sensitivity and area under the ROC curves (AUC). Further, decision trees were used to estimate the accuracy of clinical diagnosis to distinguish PIM/HI; PIMP/HI and PIMP/PIM using BOP, PI, PD, SUP and RXBL. Data mining and mathematical algorithms were performed using commercial software RapidMiner[34]. The PDM was developed using C4.5 decision trees [35]. The evaluation of predictive models was performed using Receiving Operating Characteristics (ROC) curves together with the Area Under ROC curve (AUC) representing the most popular evaluation metric in data mining and machine learning [36]

RESULTS

The final sample size consisted of 126 patients and 252 implants: HI (n=126), PIM (n=57) and PIMP (n=69). There were no statistical differences in demographic characteristics and periodontal status between PIM and PIMP patients, suggesting the inter-group homogeneity (Table 1). The comparison of clinical parameters between HI, PIM and PIMP is depicted in Figure 1. Briefly, BOP, PI and PD were significantly higher in both PIM and PIMP than in controls, SUP and RXBL were additionally increased in PIMP, while PD, SUP and RXBL were significantly higher in PIMP when compared to PM.

Bone markers between the groups

Detectability rates for RANKL and OPG were 100%, and their respective inter-group comparisons are depicted in the Figure 2. All measured biomarkers as well as RANKL/OPG were significantly higher in both PIM and PIMP compared to HI, while, surprisingly RANKL/OPG was significantly higher in PIM compared to PIMP and all other biomarkers were visibly higher in PIM as well. RANKL and OPG were highly correlated with all clinical parameters ($p<0.01$), while RANKL/OPG was positively correlated with BOP, PD and RXBL ($p<0.05$).

Diagnostic accuracy of the clinical parameters and BTMs

The accuracy, sensitivity, specificity and diagnostic ranges for standard clinical parameters and BTMs are listed in the Table 2. BOP and PI demonstrated 100% accuracy, specificity and sensitivity to distinguish PIM and PIMP from HI, when present in more than about 2/6 points for BOP and 3/6 points for PI per implant. Additionally, PD showed similarly high diagnostic accuracy to distinguish PIMP from HI when ≥ 4mm, while the accuracy and specificity of this parameter were decreased for diagnosis of PIM. SUP and RXBL were highly specific indicators of PIMP while the sensitivity and accuracy of SUP were low, since the average frequency of SUP counted about 30%. RANKL showed the 100% accuracy, sensitivity and specificity for PIM diagnosis, while the respective values were slightly lower in PIMP (95%, 92%, 96,10%). OPG showed similar trend with slightly decreased diagnostic capacity compared to RANKL. RANKL/OPG showed clearly better diagnostic properties in PIM than in PIMP although this parameter was 100% specific for PIMP.

The accuracy of the clinical diagnosis on implants

The accuracy, sensitivity and specificity of diagnosis based on standard clinical parameters together with AUC of respective predictive models is outlined in the Figure 3. Clinical parameters were the most performant to distinguish PIMP from HI (94%), their respective accuracy was lower for discrimination of PIM from HI (86.67%), while the lowest accuracy was demonstrated for discrimination of PIM and PIMP (58.55%). However, the estimation of clinical diagnosis using highly precise classification algorithms showed relatively low diagnostic effectiveness according to the low AUC for all three diagnostics (0.589-0.711) (Figure 3). Indeed, when PIM and PIMP were pooled and compared to HI, the accuracy, specificity and sensitivity of the clinical diagnosis demonstrated the highest values while the AUC of the predictive model was very high and counted of 0.913 (Figure 3).

Diagnostic capacity of the personalized diagnostic model

The PDM for implant diagnostics based on the clinical parameters and BTMs is depicted in the Figure 4. The predictive model identified BOP, PD and RANKL as critical parameters for discrimination of peri-implant conditions with high accuracy of (96.27% +/- 4.57%) sensitivity (95.00% +/- 6.31%) and specificity (100%+/-0.00%). It was confirmed that BOP < 25% clearly discriminates HI, while BOP>25% and PD>4.5mm indicated PIM. However, the PDM identified RANKL as critical parameter for diagnosis of peri-implant states associated with bone loss and related discrimination of PIM from PIMP. The PDM additionally identified second cluster of clinically diagnoses PIM characterized with similar characteristic to PIMP and exceeding levels of RANKL that might correspond to the PIMP onset or progressive form of the disease.

DISCUSSION

1.1. Principal findings

The present study confirmed BOP, PI and PD as solid discriminants between peri-implant health and inflammation, while increase in PD (PD>4mm) and SUP showed to be good discriminants between PIM and PIMP. However, PIM and PIMP could be clinically distinguished in approximately 60% cases while about 40% remained as false negatives (accuracy: 58.55%; AUC:0.589±0.177) suggesting a limitation of clinical parameters for diagnosing early PIMP. Moreover, the BMTs levels showed to be similar between PIM and PIMP, while the algorithms demonstrated two clusters of PIM patients based on the exceeding BTMs levels probably corresponding to the early PIMP. Additionally, two clusters of PIMP were identified that might possibly corresponds to the different disease grades. Finally, PDM combining clinical parameters and BTMs provided outstanding diagnostic properties with substantially improved accuracy, sensitivity and specificity when compared to standard clinical diagnosis.

1.2. Diagnostic capacity of clinical diagnosis on implants

A recent clinical and preclinical study conducted by our group elucidated on the need for using the combination of parameters to accurately diagnose peri-implant conditions instead of using individual parameters [17]. It was also highlighted that specificity that often overcomes respective sensitivity represents a leading factor that negatively impacts clinical diagnostic accuracy of implants[17,37] which was confirmed in the present study. Interestingly, it has been shown that BOP and PI showed 100% diagnostic accuracy, including the specificity and sensitivity to indicate peri-implant disease when positive in about 2 and 3 points per implants, respectively. Not surprisingly, diagnostic ranges were similar for PIM and PIMP. Moreover, the increase in PD (PD>4mm) was a highly accurate indicator (100%) of peri-implant disease, while in PIM the estimated threshold value was 2.8mm and respective

accuracy and specificity were slightly decreased when compared to PIMP. SUP and RXBL remained highly specific indicators for PIMP, however the sensitivity of these parameters was not outstanding due to low frequency for SUP counting about 30% of implants and understandable low precision of radiological bone changes. Regarding the reliability of the advanced predictive algorithms, it was estimated a good accuracy of the clinical parameters to distinguish PIMP/ HI (94%) and for PIM/HI (86.67%), while the capacity of clinical parameters to distinguish PIMP/PIM was very low (58.55%). With regard to the recently proposed classification of peri-implant conditions [16], it was confirmed that BOP, PI and PD are good discriminators between peri-implant health and disease, while it was also confirmed that increase in PD associated with RXBL is indicator of PIMP [16,38]. However, SUP that's was also proposed indicator of PIM [16] was limited to PIMP as previously reported by Ramanauskaite et al. [39].

1.3. BTMs profile between different peri-implant conditions

BTMs are fast responsive markers of bone metabolism measured using highly sensitive diagnostic assays capable to capture their respective shift in concentrations before clinically manifested changes thus overcoming the mm-level clinical measurements. However, validation of biomarkers for diagnostic use represents a comprehensive and exhaustive process conducted per rigorous guidelines [24,25,40] This research team worked on validations of biomarkers of peri-implant condition [26,27,41], and in the previous studies were confirmed suitable candidate markers while the diagnostic protocol including sampling, storage and analytics were optimized corresponding to the first stage of biomarker validation [24,25]. The use of advanced informatic algorithms is an intrinsic characteristic of biomarker validation and personalized medicine in general, since the biomarkers are validated related to standard clinical endpoints whose lack in diagnostic capacity is, actually, the reason for introducing the biomarkers. Therefore, since routine statistical tests use a linear pattern and are unable to bypass

such a challenge, the mathematical algorithms able to cross-analyze the data and identify so called "hidden knowledge" with high precision represent the method of choice in precision medicine.

The present study was carefully designed to estimate diagnostic effectiveness of RANKL and OPG according to guidelines [23,24], thus exact accuracy, sensitivity, specificity and diagnostic intervals for BTMs in implantology were estimated for the first time. The biomarkers profile between different peri-implant conditions diagnosed according to clinical case definition and based on strong correlation with standard clinical endpoints biomarkers answered requests for diagnostic markers and as these were considered suitable for the purpose of the study [24]. The BTMs showed similar profile in both PIM and PIMP characterized with significantly higher levels of RANKL, OPG and RANKL/OPG when compared to HI. Additionally, in approximately 40% of PIM, the BTMs were visibly higher in PIM while RANKL/OPG was even significantly higher in PIM than in PIMP which clearly demonstrated active bone losing in stage of PIM. Although, the accuracy, sensitivity and specificity of BTMs were generally high with tendency of slightly better diagnostic properties in PIMP, there was an overlapping in diagnostic ranges between PIM and PIMP, while the algorithms identified two clusters of patients in both conditions. The increased RANKL levels in PIM that surpass PIMP levels might be possibly explained by biological features of RANKL expressing its primary function in initiation of osteoclastogenesis followed by increase concentration when compared to the levels in established bone lesions such as PIMP, since the maintenance of activated osteoclasts represents its secondary function. In that context, the second PIM cluster might represent PIMP onset associated with initial inflammatory osteoclastogenesis. Another possibility is that second patient clusters in both PIM and PIMP might represent individuals with progressive forms of the disease which remains the most important implication for the future research.

1.4. Personalized medicine in implantology

The PDM developed on the clinical parameters and BTMs demonstrated the highest diagnostic accuracy of 96.27%, 95% sensitivity and 100% specificity, while the model performance was estimated with high AUC of 0.954. As a matter of fact, the algorithms applied, identified BOP and PD as accurate parameters for discrimination of peri-implant conditions without bone resorption. Thus, BOP <25% clearly discriminated HI, while BOP>25% and PD>4.5mm indicated PIM. However, PDM used RANKL as a critical factor to distinguish PIM from PIMP. These findings suggest that clinical parameters are reliable to discriminate peri-implant health from inflammation, but for accurate diagnosis of pathological bone loss, the adjuvant use of bone markers remain of critical importance for accurate and timely diagnosis.

1.5. Implications for the future research

The use of biomarkers in periodontology and implantology both in clinical and research setting has been subject of recommendation for a while within the concept of *"The future of Biomarkers and Personalized medicine in dental medicine"* [42–44]. Related to that, biomarkers were for the first time introduced as diagnostic criteria in the new classification of periodontal and peri-implant conditions[45], while the need for identification and standardization of biomarkers for appropriate disease grading was emphasised.[45] The present study confirmed pre-analytical and intra-analytical validity of RANKL and OPG for implant screening per implemented protocol, however the main obstacle for diagnostic standardization was mismatching between clinical case definition and biological characteristics of PIM regarding confirmed bone resorption in PIM. Moreover, the routine statistical methods allowed demonstration only of the lack in difference in bone resorption markers between PIM and PIMP, while the classifying algorithms identified cluster of PIM patients that actually represented exceeding

levels of respective markers. Therefore, the need for shifting from clinically-based towards biologically supported definition of peri-implant conditions seems to be imminent, while the personalized approach seems to be the appropriate avenue towards resolution of current *circulus vicious* with peri-implant pathologies. Thus, the well-designed prospective studies on a bigger sample are required to confirm the origin of identified patient clusters in order to set the exact threshold for PIMP onset as well as to establish the capacity of BTMs for disease grading. Additionally, as the present study evaluated only the capacity of BTMs for disease diagnosis, the future prospective studies should attempt to estimate the prognostic value of respective markers via assessing BTMs in response to performed treatment and in extended follow-up period. Moreover, it was concluded that the reason for frustratingly slow progress of biomarker standardization is the so called "sloppy science" relying on poor clinical study designs and lackig standards for specimen collection, biomarker analysis and appropriate data management[46] In the spirit of that, the future biomarker studies in implantology should be designed using more rigorous research standards and according to state-of-the-art clinical recommendations and guidelines for biomarker validation, while standard data management in implant research should be supplied by algorithm assessment.

CONCLUSION

Biomarker assessment demonstrated similar levels of bone resorption markers between PIMP and PIM that seems to be related to the identified cluster of PIM patients with exceeding BTMs levels. Hence, although the BTMs demonstrated capacity to substantially improve clinical diagnosis, there is a need for a more refined definition of peri-implant conditions according to biological characteristics not only for proper standardization of BTMs but primarily for an appropriate management of patients with peri-implant diseases.

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AUTHOR CONTRIBUTIONS

M.R. and A.M. conceived the ideas and led the writing; A.P.C. and D.V. conducted laboratorial analyses; S.R. analyzed the data; M.R. and Z.T. performed clinical part of the study.

FOOTNOTES

***-** North Carolina–Hu-Friedy, Chicago, IL, USA

†-Colorevue® probe Williams, Hu-Friedy, Chicago

‡-Biomedica Gruppe, Vienna, Austria

§- Prism 5.0, GraphPad Software, Inc., La Jolla, CA, USA

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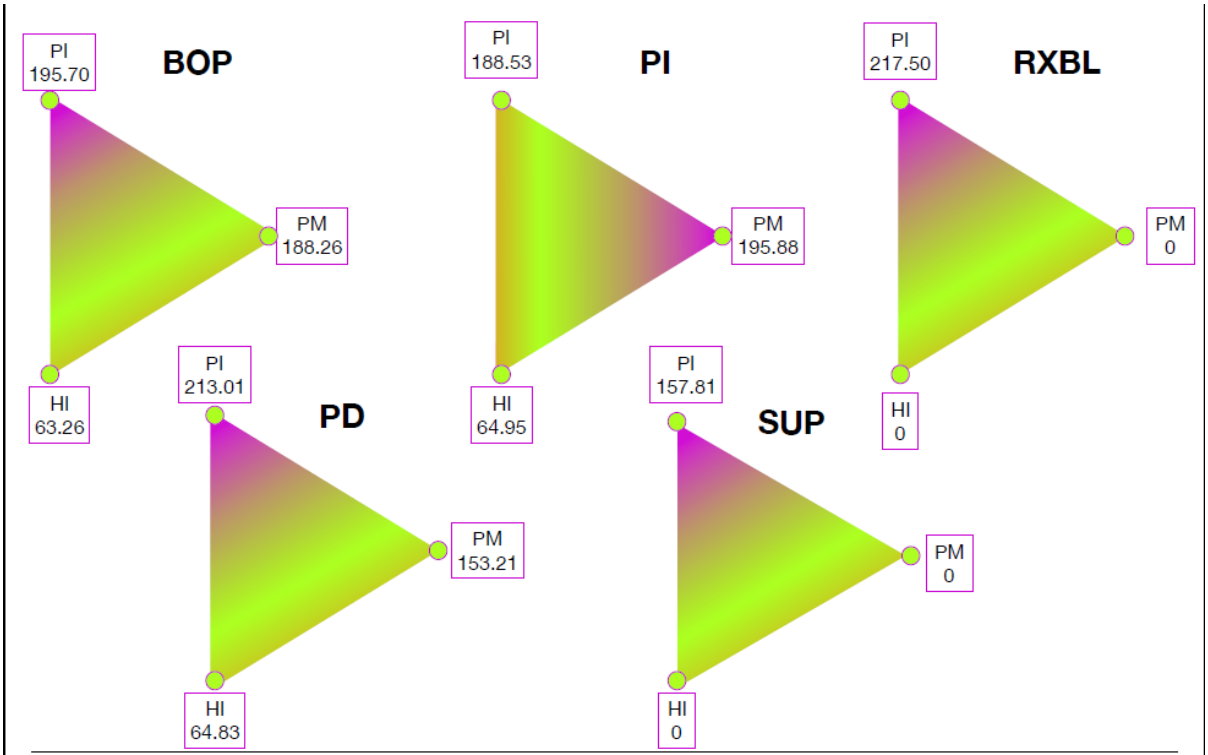
FIGURE LEGEND

Figure 1. Differences in standard clinical parameters per sites between peri-implant mucositis (PIM), peri-implantitis (PIMP) and healthy controls (HI). The diagrams depict the mean ranks of the bleeding on probing (BOP), plaque index (PI), peri-implant probing depth (PPD), suppuration (SUP) and radiological bone loss (RXBL). The asymptotic (2-tailed) significances are expressed using * in the table that summarize the differences in clinical parameters between the groups. It can be observed that BOP, PI and PD were significantly higher in both PIM and PI when compared to HI, additionally SUP and RXBL were significantly higher in PI compared to HI, while PD, SUP and RXBL were significantly increased in PI when compared to PIM.



	BOP	PI	PD	SUP	RXBL
Healthy/ Peri-implant mucositis	0.000*	0.000*	0.000*	1000	1000
Healthy/ Peri-implantitis	0.000*	0.000*	0.000*	0.000*	0.000*
Peri-implant mucositis/ Peri-implantitis	1000	1000	0.000*	0.000*	0.000*

Figure 2. Concentrations of bone loss markers between peri-implant mucositis, peri-implantitis and healthy peri-implant tissues. The diagrams depict concentration of RANKL and OPG expressed as pg/site as well as their respective relative ratio. The stars indicate that all three markers were significantly higher in peri-implant mucositis and peri-implantitis compared to healthy controls ($p<0.001$), while # additionally indicates that relative ratio was significantly higher in peri-implant mucositis than in peri-implantitis patients ($p<0.01$).

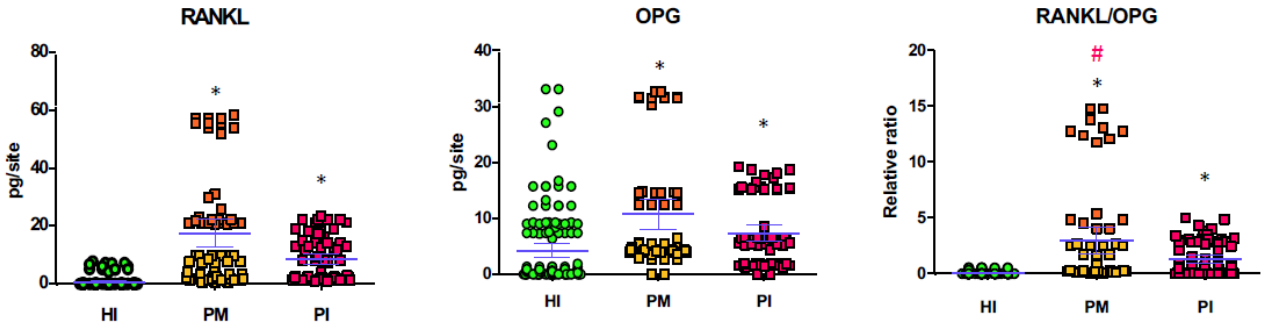


Figure 3. Diagnostic capacity of the clinical diagnosis on dental implants. The diagrams depict the capacity of clinical diagnosis to distinguish peri-implant conditions based on the BOP, PI, PD, SUP and RXBL. It can be observed that clinical diagnosis showed the lowest diagnostic accuracy to distinguish peri-implant mucositis from peri-implantitis, while indeed the pooling of peri-implantitis and peri-implantitis provided clinical diagnosis with the highest accuracy.

Figure 4. Personalized diagnostic model for implant diagnostics based on clinical parameters and bone markers. The present predictive model with accuracy rate of 96.27% +/- 4.57%, sensitivity of 95.00% +/- 6.31% and specificity rate of 100%+/-0.00% shows that BOP less than 25% clearly discriminates healthy peri-implant tissues, while BOP>25% and PD>4.5mm indicates peri-implant mucositis, while when PD overpassed 4.5mm with RANKL ≤ 19pg/site the condition represented peri-implantitis. The algorithms identified RANKL levels below 19pg/site as discriminant between peri-implantitis and second excessive cluster of peri-implant mucositis.

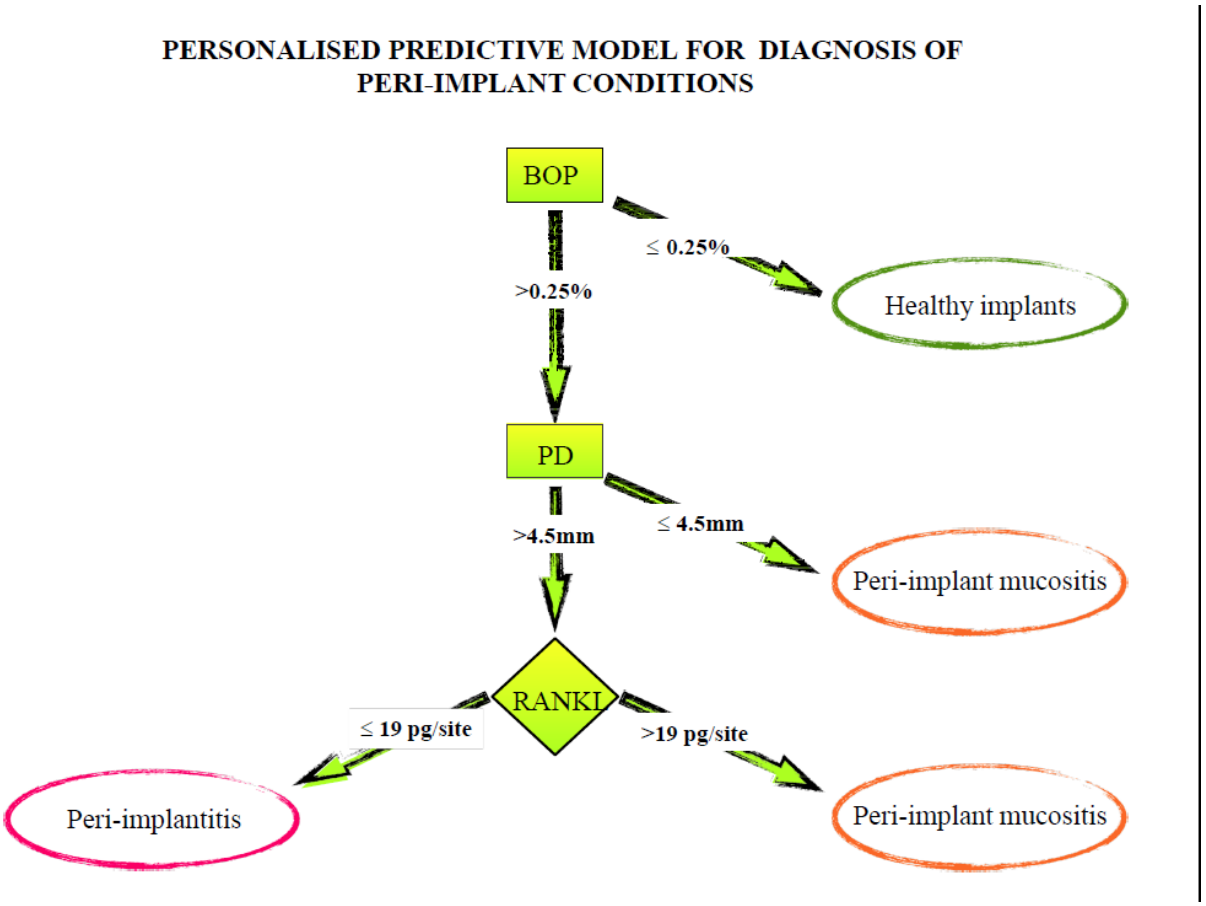


Table 1. Demographic and periodontal characteristics of the groups

Parameters	Peri-implant mucositis n=57	Peri-implantitis n=69
Gender		
Females (n)	31	32
Male (n)	26	37
Age (years; mean and range)	52.5 (24-60)	53.14 (32-68)
Number of teeth (n; mean and range)	18.9(5-25)	17.5(0-25)
Full-mouth dental plaque index (% mean ±SD)	18.4 ±10.1	25.3 ± 3.9
Full-mouth dental bleeding on probing (% means ± SD)	17.0 ±5.3	20.5 ± 4.5
Full-mouth dental probing depth (mm; mean ± SD)	3.6 ± 0.9	4.1 ± 0.7
Full-mouth CAL (mm; mean ± SD)	2.9±1.1	3.2±0.7

There were no significant differences in measured parameters between the groups.

Table 2. The accuracy, sensitivity and specificity of the individual standard clinical parameters and BTMs

Parameter	Accuracy		Sensitivity		Specificity		Diagnostic range	
	Peri-implant mucositis	Peri-implantitis	Peri-implant mucositis	Peri-implantitis	Peri-implant mucositis	Peri-implantitis	Peri-implant mucositis	Peri-implantitis
Clinical parameters								
BOP	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 +/ 0.00	100 ± 0.00	100± 0.00	D >25% H ≤ 25%	D >25% H ≤ 25%
PI	100± 0.00	100 ± 0.00	100± 0.00	100.00 ± 0.00	100± 0.00	100± 0.00	D >50% H ≤ 50%	D >50% H ≤ 50%
PD	94.45 ± 7.22	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	87.27± 28.10	100± 0.00	D >2.8mm H ≤ 2.83mm	D >4.0mm H ≤ 4.0mm
SUP	-	68.9± 21.00	-	33.00 ± 16.80	-	100± 0.00	-	D >16.66% H =0 %
RXBL >2mm	-	100 ± 0.00	-	100 ± 0.00	-	100 ± 0.00	-	D >2mm H ≤ 2mm
Bone turnover markers								
RANKL	100± 0.00	95.00 ± 12.81	100 ± 0.00	92.00 ± 20.00	100 ± 0.00	96.10± 6.47	D >11.0 H ≤ 1.095 *D 1.096-6.984*	D >8.0 H ≤ 0.594 *D 0.6-4.817*
OPG	96.67 ± 6.67	94.00 ± 18.00	94.53 ± 14.00	95.00 ± 16.20	100 ± 0.00	92.73± 8.63	D >22.0 H ≤ 2.060 *D 2.060-7.373*	D >11.0 H ≤ 1.095 *D 1.096-6.984*

RANKL/OPG	95.00 ± 7.64	64.00 ± 11.09	96.40 ± 12.56	45.00 ± 18.30	100 ± 0.00	100 ± 0.00	*D*>1.21 *H* ≤1.21	D >1.1 H ≤ 1.1

- *0 or unsuitable for interpretation; In italic*- second level diagnostic range