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Diagnostic value of VEGF in peri-implantitis and its correlation with titanium particles: A controlled clinical study

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ABSTRACT

Objectives: VEGF is prototypic marker of neovascularization, repeatedly proposed as intrinsic characteristic of peri-implantitis. This study aimed to assess pattern of VEGF in peri-implantitis, its correlation with titanium particles (TPs) and capacity as respective biomarker.

Material and methods: Pathological specificity of VEGF was assessed in peri-implant granulations using immunohistochemistry, periodontal granulations represented Ti-free positive controls. VEGF was correlated to TPs, identified using scanning electron microscopy coupled with dispersive x-ray spectrometry. Diagnostic accuracy, sensitivity and specificity of VEGF were estimated in PICF specimens from peri-implantitis, peri-implant mucositis (PIM) and healthy peri-implant tissues (HI) using machine learning algorithms.

Results: Peri-implantitis exhibited rich neovascular network with expressed density in contact zones toward neutrophil infiltrates without specific pattern variations around TPs, identified in all peri-implantitis specimens (mean particle size 8.9 ± 24.8 μm^2; Ti-mass (%) 0.380 ± 0.163). VEGF was significantly more expressed in peri-implantitis (47,065 ± 24.2) compared to periodontitis (31,14 ± 9.15), and positively correlated with its soluble concentrations in PICF ($p = 0.01$). VEGF was positively correlated to all clinical endpoints and significantly increased in peri-implantitis compared to both PIM and HI, but despite high specificity (96%), its overall diagnostic capacity was average. Two patient clusters were identified in peri-implantitis, one with 8-fold higher VEGF values compared to HI, and second with lower values comparable to PIM.

Significance: VEGF accurately reflects neovascularization in peri-implantitis that was expressed in contact zones toward implant surface without specific histopathological patter variation around TPs. VEGF answered requests for biomarker of peri-implantitis but further research is necessary to decrypt its exact underlying cause.

1. Introduction

Peri-implantitis is qualified emerging public health problem due to its increasing prevalence and lack of predictive treatment, that becomes burdensome with continual expansion of the implant market [1,2]. Dental implants are undeniable gold standard treatment for replacement of missing teeth, however the specific factors in oral environment sch as abundant microflora, expressed physicochemical variations and strong biomechanical forces contribute to the frequent advent of peri-implant complications. Peri-implantitis referring to the chronic inflammatory

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disease of the peri-implant tissues induced by infection represents the most frequent complication of dental implants. It is estimated that peri-implantitis affects between 20% and 50%, while the lack of predictive treatment results in 50–100% recurrence rate within first two years post-treatment [3,4]. Such unpredictable treatment responsiveness on treatment protocols adopted form periodontology has stimulated in-depth comparative research between periodontitis and peri-implantitis and it is now established that despite shared etiology those diseases exhibit critical differences in pathological patterns [5,6]. Peri-implantitis shows more aggressive inflammatory behavior than periodontitis, non-linear pattern of progression and asymptomatic course so it is considered that histological specificities of the peri-implant issues and implant-related factors plays the role in such specific pathological pattern [2]. In the spirit of that, the major efforts are invested in identification of the biological targets underlying such specific pathological pattern for related improvement of the diagnostic and treatment protocols in management of the implant patients. In the sprite of such alerting epidemiological numbers and pathological pattern behind peri-implantitis, the early and accurate diagnosis of peri-implantitis is defined as ultimate priority and decisive factor for treatment success [7]. Standard diagnostic protocols in implantology exhibit substantial limitations in providing timely and accurate diagnostic information [8,9], and indication concerning implication of the specific biological factors which is why the personalization of the clinical strategies in periodontology and implantology is defined as ultimate priority [8–10].

Aseptic peri-prosthetic osteolysis (PPO) is the most frequent cause of implant loosing and revision surgeries in orthopedics [11,12] and TPs are considered as major culprit behind this pathology which is why Dr. William Harris termed it as particle disease [13]. While the TPs are thoroughly investigated in orthopedics, this remains a burgeoning field in dentistry, so the emerging concept about the role of TPs in peri-implantitis was proposed recently [14]. The major efforts were invested in deciphering possible underlying molecular mechanisms of TPs in peri-implantitis [15] and in vitro studies have demonstrated the enhancing pro-inflammatory effects of the TPs particularly when exposed to the lipopolysaccharide [16,17]. Moreover, it was reported that Ti ions may deteriorate peri-implant inflammation and alveolar bone resorption by increasing susceptibility of the epithelial cells to Porphyromonas gingivalis (the key stone pathogen in peri-implantitis) and enhancing the osteoclast differentiation and activity via RANKL upregulation [18]. The human studies so far demonstrated the presence of TPs in granulation tissue from peri-implantitis [19–21] but without establishing any specific pathological pattern to TPs [22]. In the recently reported study was confirmed the presence of TPs in all peri-implantitis specimens that exhibited significantly more expressed neovascularization and more intense M1 macrophage response, but no specific pathological pattern could be associated to TPs [19]. In fact identification of the specific pathological effect of TPs in peri-implantitis remain challenging since most of proposed candidate markers in orthopedics (such as IL-1, IL-6, TNFa and CD68) remain initially increased due to infection-induced chronic inflammation [23]. Expressed neovascularization was repeatedly reported as intrinsic characteristic of peri-implantitis lesion [5,19]. Neovascularization sustains inflammation, supplying the oxygen and nutrients for cellular metabolic needs within inflamed site, while increasing the afflux of inflammatory cells and mediators for more effective elimination of pathogenic insult [24]. Vascular endothelial growth factor (VEGF) is a prototypical direct-acting angiogenic factor and the most repurposed biomarker of neovascularization in in vitro diagnostics (IVD) [25]. VEGF/VEGF-receptor axis is triggered by local inflammation, while unresolving chronic infection and hypoxia further stimulate neovascularization, followed by formation and growth of granulation tissue on count of host tissue destruction [26]. This is why VEGF is frequently used as indicator of disease severity in monitoring of the inflammatory pathologies [27]. But VEGF was also proposed as biomarker of TPs induced PPO in orthopedics, based on direct correlation between VEGF levels and exposure to the TPs [28,29].

Thus, the working hypothesis was that VEGF exhibits specific pathological pattern in peri-implantitis that might be associated with TPs, while its respective soluble form in PICF may accurately reflect peri-implantitis.

Objectives of the study were: 1) to assess pathological specificity of the VEGF pattern and its correlation with TPs in granulation tissues from peri-implantitis; 2) to estimate diagnostic capacity of the soluble VEGF in peri-implant crevicular fluid (PICF) as a biomarker of peri-implantitis.

2. Materials and methods

2.1. Study design

This study was designed as cross-sectional case-control study estimating diagnostic capacity of the VEGF as a biomarker of peri-implantitis (Fig. 1). The study was conducted in two stages. First part was histological assessment aiming at assessment of the pathological specificity of the VEGF pattern in granulation tissue form peri-implantitis and identification of the TPs. The second part was clinical validation of the diagnostic capacity of the soluble VEGF in the samples of PICF (being the standard diagnostic specimen for biomarker assessment in implantology) from patients with healthy peri-implant tissues, peri-implant mucositis and peri-implantitis. The study was designed and conducted according to guidelines for validation of the biomarkers for clinical use [30] using statistical methods and machine learning algorithms, while clinical parameters represented standard endpoints.

2.2. Study population and criteria

Systemically healthy non-smokers according to American Society of Anesthesiologists classification (ASA-1) and patients with mild controlled systemic diseases and/or smoking up to 10 cigarettes per day (ASA-2) attending the Clinic for Maxillofacial, Oral Surgery and Implantology, Military Medical Academy, Belgrade, Serbia from September 2012 until November 2022 were included in the study if presenting either peri-implantitis, PIM, HI, or severe periodontitis. Final sample consisted of 148 participants including 36 peri-implantitis, 36 PIM, 39 HI and 37 periodontitis affected patients that were matched regarding clinical and demographic characteristics including gender age, smoking and periodontal status (Table 1). Conditions were defined following measurement of the clinical parameters, including plaque index (PI), bleeding on probing (BOP), probing depth (PD) and relative clinical attachment level (rCAL) that were measured in six points using light force and scored into evidential charts, according to the case definitions [31,32] as follows:

- Peri-implantitis: PD $\geq$ 5 mm, bleeding on probing (BOP)$>$ 25% and radiographic bone loss (RXBL) $\geq$ 2 mm measured from the implant shoulder to the first detectable bone to implant contact;
- PIM: with BOP $>$ 16% (positive in $>$1 point), probing depth (PD) $>$ 3 mm and RXBL $<$ 2 mm;
- HI: negative BOP or BOP positive in one-sixth sites being considered the consequence of trauma, with PD $<$ 3 mm and without evidence of RXBL from moment of loading.
- Periodontitis: presence of $>$ 2 interproximal sites with rCAL $>$ 6 mm and $>$ 1 interproximal site with PD $>$ 5 mm not on the same teeth.

Clinical parameters including PI, BOP, PD and rCAL were measured in six points using light force and scored into evidential charts. Diagnostic specimens were collected from one representative implant-site per patient, in case of $>$ 1 implant with comparable clinical parameters, 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. Diagnostic

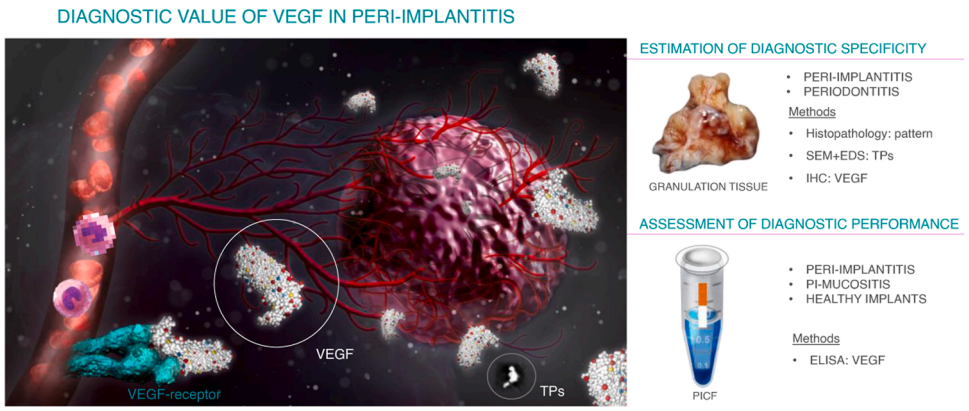


Fig. 1. Study design. Vascular endothelial growth factor (VEGF) is prototypical angiogenic factor, while the VEGF/VEGF-receptor axis is triggered by local inflammation, which is why this marker is within most repurposed diagnostic markers in vitro diagnostics of inflammatory pathologies. VEGF increases vascular permeability for improved supply of metabolically active cells within inflamed site, and enhanced afflux of immune cells, while stimulating formation and growth of granulation tissue on count of host tissue destruction. It has been hypothesized thus that VEGF accurately reflects peri-implant pathology, and that VEGF concentrations in peri-implant crevicular fluid (PICF) samples may present highly accurate biomarker of peri-implantitis. This is the 3D scientific illustration schematically portraying the role of VEGF in inflammatory pathologies and its potential applicability in in vitro diagnostics of peri-implantitis.

Table 1
Demographic and periodontal characteristics of the groups.

	Healthy implants n = 39	Peri-implant mucositis n = 36	Peri-implantitis n = 36	Periodontitis n = 37
Gender				
Females (n)	18	15	16	18
Male (n)	21	19	23	17
Age (years; mean and range)	49.5 (25–57)	53.2 (35–61)	52.5 (24–60)	53.14 (32–62)
Smokers (n)	12	16	18	21
Number of teeth (n; mean and range)	18.4 (10–25)	16.8 (0–25)	16.1 (0–25)	18.2 (6–25)
Plaque index (% mean ± SD)	11.23 ± 10.01*	80.76 ± 23.41	81.88 ± 18.31	85.7 ± 15.4
Bleeding on probing (% mean ± SD)	0 ± 0*	82.19 ± 21.34	97.1 ± 15.2	96.25 ± 12.45
Probing depth (mm; mean ± SD)	2.3 ± 1.7*	4.2 ± 2.52 **	6.1 ± 1.9	7.8 ± 3.81
Suppuration (n and %)	0 (0)**	0 (0)**	12 (30.76)	6 (17.14)
(Relative) clinical attachment level (mm; mean ± SD)	0 ± 0***	0 ± 0**	7.2 ± 3.5	8.1 ± 2.7

* - significantly lower values compared to peri-implant mucositis and peri-implantitis
** - significantly lower values compared to peri-implantitis

specimens were collected in two stages; PICF was sampled before the treatment while the granulation tissue was collected intra-surgically in case of peri-implantitis and periodontitis. The study was conducted in accordance with the Helsinki declaration for human studies, following approval of institutional ethics committee, and patient acceptance to participate by signing an informed consent.

2.3. Data management

In lack of validated VEGF diagnostic ranges, the sample size calculation was performed based on RANKL values from the previous studies on peri-implant conditions [9,33], accordingly sample size of 36 participants per group using α of 0.05 would result in a power of 0.95. The proportion of IHC positive cells per sample were expressed as median ± standard deviation and were further compared using Mann Whitney U test between the PI and periodontitis. Concentration of soluble VEGF was compared between peri-implantitis, PIM, and HI using Kruskal-Wallis test and evaluated using Mann-Whitney test, with p-values were adjusted using Dunn post-hoc test ($p < 0.016$ was considered as significant). Correlations between tissue and soluble VEGF concentrations, as well as with clinical parameters were assessed using Spearman rank correlation test. Diagnostic capacity of VEGF to discriminate different peri-implant conditions with respective diagnostic ranges was assessed using C4.5 decision trees [34], while the diagnostic performance parameters (accuracy, sensitivity and specificity were estimated using logistic regression. Area under ROC curve (AUC) was used for predictive model evaluation. Data analysis was performed using commercial softwares (SPSS v.25.0; SPSS Inc., Chicago, IL, USA; MATLAB, MathWorks, Natick, MA, USA). The present study was reported according to the STROBE guidelines.

2.4. Histological specimens and immunohistochemistry

Granulation tissue harvested during routine surgical treatment of peri-implantitis/periodontitis represented diagnostic specimen, that was stored in containers with 3.5% buffered formalin (Tissue-Tek Paraform Sectionable Cassette System by Sakura Finetek Europe, Netherlands) and immediately transported for paraffinization, while the biopsies were placed with the dissection plane oriented towards the bottom of the cassette. To avoid false-positivity, only the Ti-free scalpel and microtome blades were used for serial sections intended for histological analyses and identification of TPs, the way that the first and last (medial) peri-implantitis sections were used for dispersive X-ray spectrometry (EDS), while the forthcoming sections and all periodontitis specimens were processed for standard and immunostaining (IHC). Sections were stained with haematoxylin-eosin or IHC using commercially available antibodies for VEGF (1:100, sc-7269, Santa Cruz Biotechnology, USA) and IHC detection kit (DAKO; Dako Dual Endogenous Enzyme Block; EnVision System- HRP; DAB, DakoCytomation, Glostrup, Denmark) following standard diagnostic protocols and manufacturer's instructions. Slides were counterstained with haematoxylin, manually dehydrated, mounted and cover slipped. Sections were examined by an experienced blinded pathologist using bright-field microscopy (BX50 Olympus, Inc, Japan) equipped with imaging system (Q-500 MC; Leica, Wetzlar, Germany). VEGF-positive cells were quantified using NIH ImageJ software (NIH, Bethesda, Maryland; https://imagej.nih.gov/ij/) in aid of IHC profiler plugin [35]. VEGF was quantified in 4–5 fields/sample (300 × 200 μm) at x40 magnification, values were averaged per sample and expressed as proportion of positive cells. IHC

quantification was performed by two blinded examiners following calibration on 10 randomly selected immune- stained section (inter--examiner κ: 0.981; intra-examiner κ D.V.: 0.974, M.R.:0.988).

2.5. Identification of TPs

Metal particles were identified in medial superficial and medial sections of peri-implantitis samples to avoid false positivity, using polarized microscopy and scanning electron microscopy (Leo 1450 VP, Zeiss, Oberkochen, Germany) [36,37]. Carbon metallization was applied to avoid interference with metals, specimens were analyzed using accelerating voltage (20 kV) and varying magnifications for identification of particle-like structures and scanned with a 100 μm x 100 μm beam size. Regions with identified particles were magnified and characterized using dispersive x-ray spectrometry (EDS) to confirm elemental composition. Between 5 and 10 spectres per sample were analysed and elemental composition was expressed as mean mass percentage of elements (mass %) of the tissue surface under the sonde by spectre (100 μm × 100 μm beam size) at varying magnifications. The analysis was performed by one experienced engineer, while the same calibration principle as for the histopathological assessment was performed (intra-examiner κ: 0.973).

2.6. PICF sampling and measurement of soluble VEGF concentrations

PICF represented diagnostic specimens for measurement of VEGF, that was sampled, stored and processed according to the previously reported protocol [38]. In brief, 24–72 h post-examination the samples were retrieved using filter technique by placing the standardized paper strips till mild resistance for 30 s at the mesial sites, stored in micro-centrifuge plastic tubes containing 0.5 mL sterile phosphate-buffered saline, and transported for analysis. VEGF was measured using commercial enzyme-linked immunosorbent assay (Human VEGF ELISA Development Kit, Promokine, PromoCell GmbH, Heidelberg, Germany) and expressed as total biomarker amount (ng) per site in 30 s [39].

3. Results

Demographic and clinical characteristics of the groups are provided in the Table 1.

3.1. Neovascularization, VEGF tissue expression and its correlation with TPs

All peri-implantitis and periodontitis specimens showed chronic inflammatory infiltrate interweaved with neutrophil infiltrates that were histopathologically diagnosed as chronic inflammation induced by bacterial infection, while peri-implantitis specimens showed typical lymphocyte infiltrate dominated with plasma cells, that varied from chronic to sub-acute form with focal neutrophil and eosinophil infiltrates. Peri-implantitis demonstrated dense neovasculature characterized with hyperaemic vessels, frequently associated with micro-bleeding and focal erythrocyte infiltrates (Fig. 2). Periodontitis expressed scarce VEGF positivity, while peri-implantitis granulations were highly positive on VEGF with significantly higher proportion of VEGF+ cells (periodontitis= 31,14 ± 9.15; PI= 47,065 ± 24.2; $p = 0.021$) (Fig. 3). All peri-implantitis specimens were Ti+ with TPs observed as sparse spot-like (Fig. 4, A) or solitary particles (Fig. 4, B), with mean particle size 8.9 ± 24.8 $\mu m^2$and Ti-mass (%) 0.380 ± 0.163. TPs were observed as free content embedded in granulation tissue, while the multinucleated giant cells (MNGCs) or frustrated phagocytes were not observed in any specimens indicating no signs of foreign body reaction or any specific pathological effect of TPs in peri-implantitis.

3.2. Diagnostic capacity of VEGF

Diagnostic capacity of VEGF in PICF samples is outlined in Fig. 5. Soluble VEGF concentrations were positively correlated to tissue VEGF expression ($p = 0.01$) and were significantly higher in peri-implantitis when compared to PIM and HI. VEGF was positively correlated to all clinical endpoints (Fig. 5). Diagnostic capacity of VEGF to distinguish peri-implant conditions was estimated as modest with accuracy: 51.74% + /- 14.39%, sensitivity: 37.33% + /- 30.47% and specificity: 58.04% + /- 13.48%. Predictive model was performant in distinguishing cluster of peri-implantitis with exceeding values (VEGF>8.896 ng/mL) from HI (VEGF<0.208 ng/mL), while respective diagnostic performance declined for discriminating cluster of peri-implantitis with lower VEGF (VEGF<8.896 ng/mL) from PIM due to great-deal overlapping in

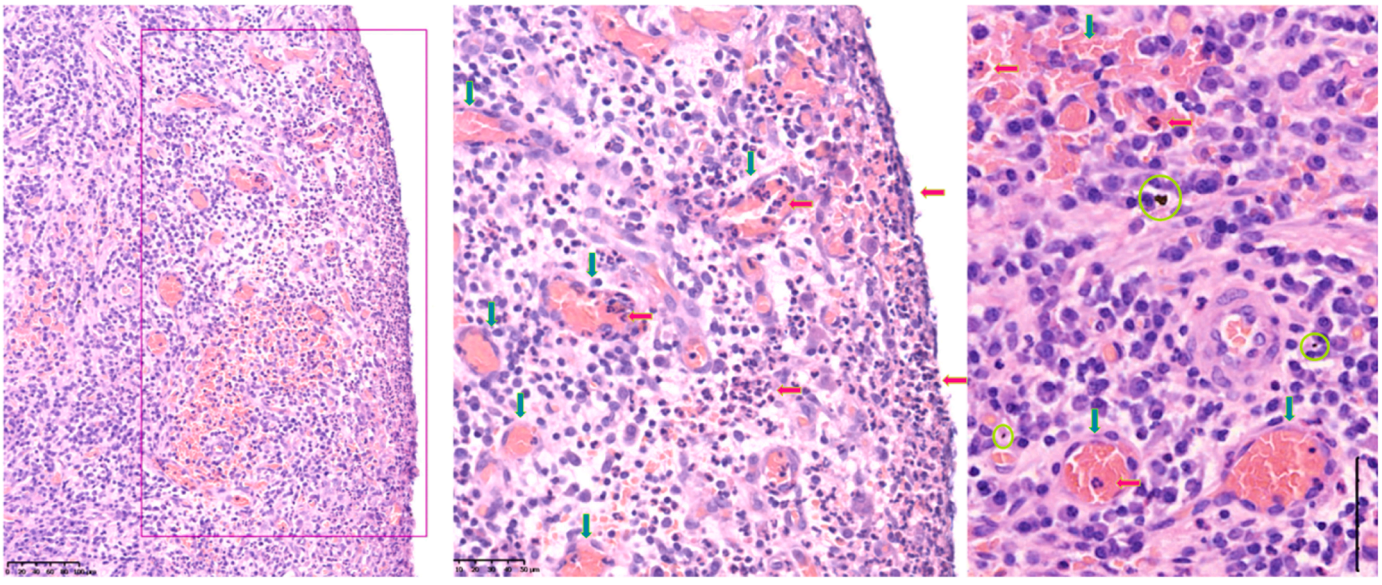


Fig. 2. Pattern of neovascularization in peri-implantitis. Dense zone of neovasculature has been observed adjacent to the neutrophil infiltrates in contact zone toward implant surface (A). Vessels were hyperemic with zones of micro bleeding (B, C, green arrows), while the neutrophil diapedesis could be observed in endothelium of hyperemic vessels (red arrow, B), as well as in lumens of hyperemic vessels and extravasated blood content (C, red arrows). Titanium particles (C, in green circles) were observed as a free content without pathological signs of specific immunological response against them or densification of neovasculature in their vicinity.

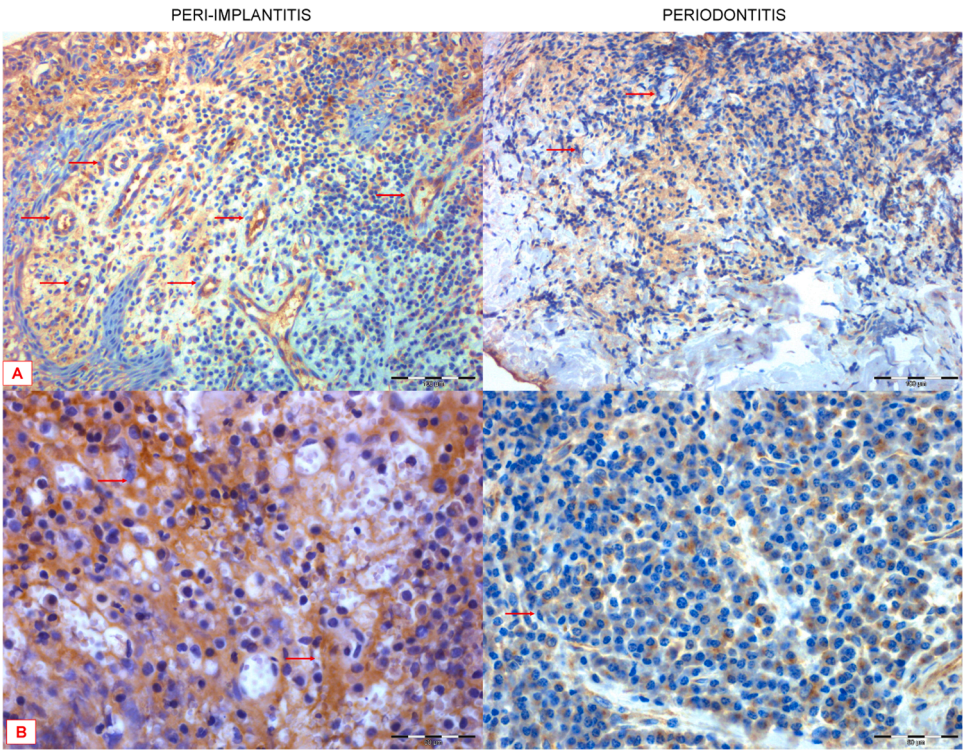


Fig. 3. Tissue expression of VEGF between peri-implantitis and periodontitis. VEGF was significantly more expressed on the neovasculature (A) and the cells (B) in peri-implantitis in contrast to periodontitis that exerted less expressed neovascularization and scarce VEGF-positivity characterized with visibly less intensive staining. Intensive VEGF expression on endothelial cells (A, red arrows) is characteristic for newly formed and activated neovasculature. Proportion of total positive cells ($p = 0.021$) was significantly higher in peri-implantitis when compared to periodontitis. Figure depicts VEGF-stained granulation tissue from peri-implantitis, and periodontitis captured at x20 (B) and x40 magnifications (A).

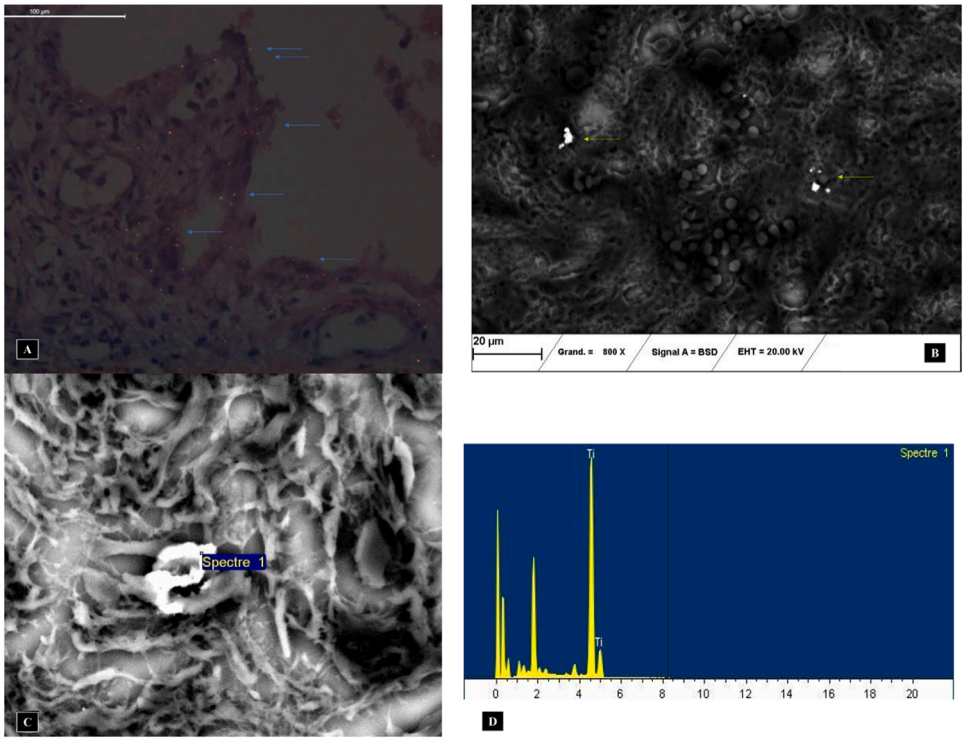
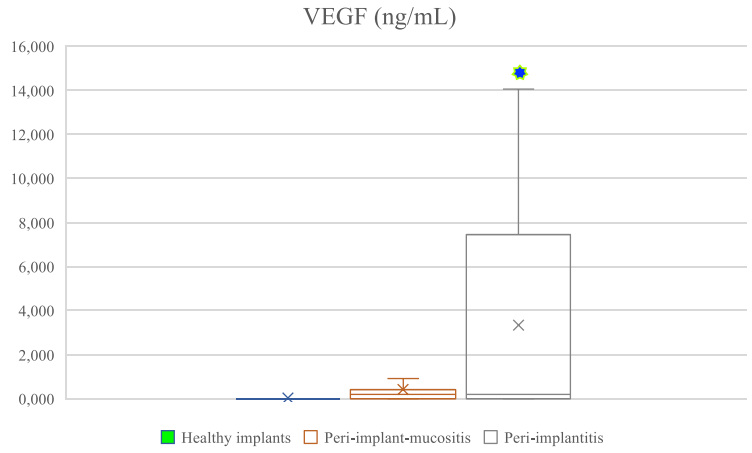


Fig. 4. Identification of titanium particles (TPs) in granulation tissues. Metal-like particles were identified using polarized light (A) and scanning electron microscopy (B). Identified metal-like particles were further inspected under varying magnifications (C), while the elemental composition was established using dispersive x-ray spectrometry, while the remaining to confirm that metal particles are TPs (D). TPs were confirmed in all peri-implantitis specimens in form of dispersed spot-like pattern (A) or as larger solitary particles (B). TPs were not identified in periodontitis.

A



B

Diagnostic capacity	Healthy peri-implant tissues		Peri-implant mucositis		Peri-implantitis
Accuracy	70.00% +/- 12.51%		67.86% +/- 16.47%		71.43% +/- 13.47%
AUC	0.746 +/- 0.177		0.617 +/- 0.193		0.746 +/- 0.119
Sensitivity	59.17% +/- 32.26%		34.17% +/- 29.25%		45.83% +/- 21.96%
Specificity	80.00% +/- 14.27%		95.00% +/- 10.54%		96.67% +/- 10.54%
Correlations with tissue expression and clinical endpoints					
VEGF	**Tissue VEGF**	**PD**	**BOP**	**PI**	**rCAL**
Correlation coefficient (p-value)	.361** .000	.364** .000	.301** .001	.310** .001	.275** .003

C

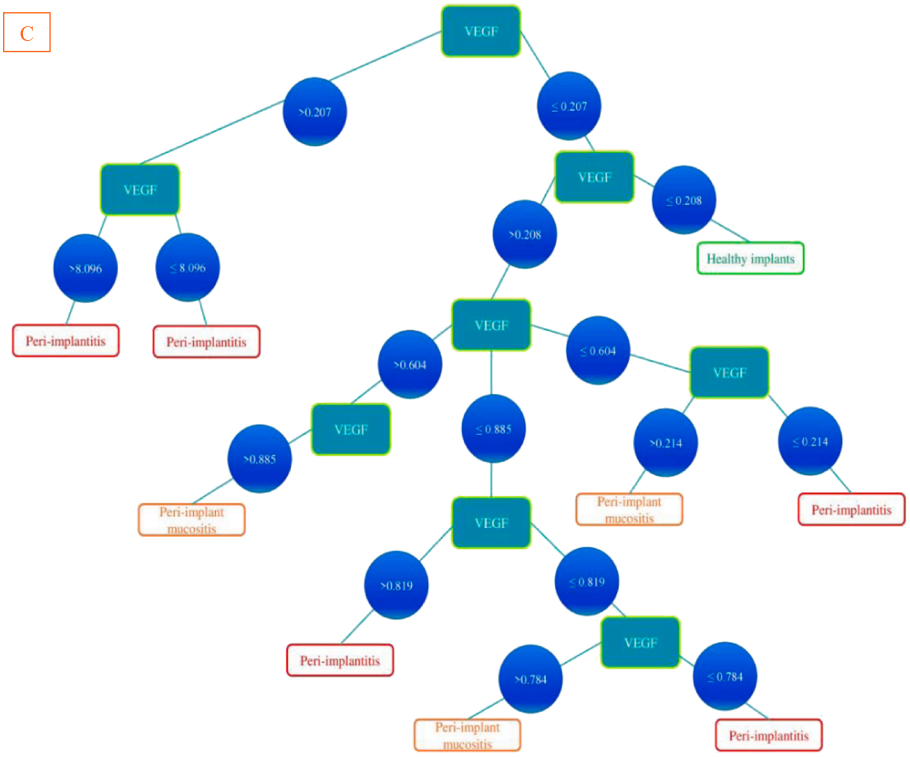


Fig. 5. Diagnostic capacity of soluble VEGF for peri-implantitis diagnostics. Diagnostic capacity of VEGF was estimated by comparing its respective concentrations between peri-implant conditions by means of standard statistical tests (A). Its diagnostic performance parameters including accuracy, sensitivity and diagnostic ranges were estimated using machine learning algorithms (B, C). VEGF concentrations were significantly higher in peri-implantitis ($p = 0.001$) compared to both peri-implant mucositis and healthy peri-implant tissues ($p > 0.05$) (A). Diagnostic performance parameters of VEGF showed its high specificity to peri-implantitis (96.65) but modest capacity to discriminate peri-implant conditions which was particularly expressed in distinction between peri-implantitis and peri-implant mucositis (B). Decision trees (C) identified two clusters of peri-implantitis, and predictive model was more performant in diagnosing cluster of peri-implantitis with remarkably increased VEGF (>8.896 ng/mL) and healthy implants (<0.208 ng/mL), while respective capacity declined for discriminating peri-implantitis cluster with lower values (<0.207 ng/mL) from peri-implant mucositis. Predictive model performance was as follows; accuracy: 51.74% + /- 14.39%, sensitivity: 37.33% + /- 30.47%, specificity: 58.04% + /- 13.48%.

concentrations. VEGF was highly specific to peri-implantitis (96,67%), with higher performance in distinguishing peri-implantitis /HI (AUC: 0.746 +/- 0.177) than peri-implantitis /PIM (AUC: 67.86% +/- 16.47%), although the sensitivity and overall biomarker diagnostic capacity was average.

4. Discussion

The present study confirmed expressed neovascularization as intrinsic characteristic of peri-implantitis and demonstrated the capacity of VEGF to convincingly reflect it. VEGF did not show specific pathological pattern around TPs that were identified in all peri-implant granulations, while the dense neovasculature was observed around neutrophil infiltrates in the contact zone toward implant surface. Soluble levels of VEGF in the PICF were positively correlated to the tissue VEGF levels confirming the capacity of the VEGF measurement in the PICF to reliably reflect peri-implant neovascularization.

Histopathological studies have demonstrated that peri-implantitis and periodontitis exert critical pathological differences while the larger lesion size, lymphocyte-chronic infiltrate dominated by plasma cells and expressed neovascularization were established as pathological characteristics of peri-implantitis [5] which was confirmed in the present study. Vascular network density was visibly more expressed in peri-implantitis compared to periodontitis and represented by hyperemic vessels and zones of micro-bleeding which was quantitatively confirmed by significantly increased VEGF expression in peri-implantitis. In context of significantly higher VEGF in per-implantitis compared to periodontitis that already exhibits significantly increased VEGF than healthy periodontium [40], these findings are confirmative that neovascularization represents an intrinsic characteristic of peri-implantitis and suitability of this marker to reflect it. The expressed neovascularization in the contact zones toward implant surface behind neutrophil infiltrates was dominant pathological specificity observed in histopathological profiling. Two plausible causes may underly such finding. First, the neutrophil infiltrates in the contact zone are suggestive for anti-infective inflammatory response on biofilm-contaminated implant surface. In that sense, the reactive neovascularization to the intensive inflammatory metabolism in zone of anaerobic infection may be possible explanation. Microbe-leaded neovascularization is usual finding in chronic infections due to local hypoxia that steadily upregulates VEGF further promoting neovascularization, while the positive correlation between VEGF in PICF samples and *Treponema denticola* loads in peri-implantitis have been reported [41]. *In vitro* studies demonstrated the capacity of TPs to dysregulate antigen-presenting pathways [42] and to upregulate production of pro-inflammatory cytokines in lipopolysaccharide-primed macrophages [16]. Recent research also reveals the cross-interaction between TPs, peri-implant microbiome and related host response as plausible explanation behind more complex and different pathological pattern in peri-implantitis when compared to periodontitis [43,44]. In that context, cytochemical effects of TPs may represent another cause or rather contributing factor in more expressed neovascularization observed in peri-implantitis.

TPs are common finding around titanium implants most commonly originating from drilling during implant placement surgery, fretting at abutment connections under biomechanical forces, professional dental instrumentations (such as scaling) and in response so to the antiseptics with corrosive properties [45–48]. Biological response to TPs is defined by particle size, shape, and material properties, and depends on local factors such as pH and oxygen tension that affect passivation of the particles and reconstruction of the biocompatible oxide layer [45]. In case of infection-induced inflammation such as in peri-implantitis, the acid environment stimulates dissolution of the oxide layer and subsequent TPs release, while preventing reoxidation of the particles which may lead to the cytochemical interaction between titanium and immunological networks with deterioration of the local inflammation. In brief, TPs upregulates activity of monocytes and M1 macrophages with subsequent increase of pro-inflammatory cytokines implicated in peri-implant inflammation such as IL1, IL-6 and TNFa. More specifically, TPs directly stimulate inflammatory bone resorption by upregulating RANKL and osteoclast activity while suppressing bone formation [49]. However, most of these finding originate from in vitro studies, hence the unestablished pathological threshold for titanium concentration in biospecimens, the lack of valid diagnostic indicators and method for respective evaluation represent once of the most concerning aspects in implantology and orthopedics [50]. While initial studies focused on presence of MNGCs as indicator of reaction on TPs, the current focus is on identification of the cytochemical pro-inflammatory effects of TPs [12,16] since it is considered that MNGCs occurs in the end-stage chronic inflammatory response on TPs [51,52]. Additionally, the recent studies suggest that FBR-MNGCs and osteoclasts cannot be accurately discriminated at the bone-to-implant surface without appropriate characterization [53]. Identification of the independent pro-inflammatory effects in human studies in case of dental peri-implantitis is challenging given the fact that in contrast to aseptic PPO, peri-implantitis relies on established infection-induced inflammation and most of proposed PPO markers such as IL-1, IL-6 and TNFa remain already increased. This was indirectly confirmed in the previous study reported by this research team, demonstrating that despite clear histopathological differences peri-implantitis and periodontitis exhibited similar pattern and intensity of inflammation based on comparable levels of IL6 and nuclear factor kappa-b, while CD68 and VEGF were significantly higher in peri-implantitis [19]. In fact, this study served as a pilot study motivating the extension of the research performed in the present study since VEGF was the single marker presenting the distinctive pathological pattern between peri-implantitis and its counterpart on teeth. Additionally, VEGF was proposed as biomarker of TPs-induced PPO in orthopedics based on its pattern of direct increase in response to TPs exposure. TPs were identified in 100% of peri-implantitis specimens in different shape and size, with predominant appearance of larger solitary particles in the central granulation portions, and smaller spot-light particles and ions in the contact boundary zone toward implant surface. The particles were observed as a free content without any signs of cellular agglomeration, frustrated phagocytosis, or foreign body reaction-type multinucleated giant cells (FBR-MNGCs) which is in accordance with reported studies with exception of the presence of MNGCs in 10% of peri-implantitis reported by Wilson et al.[20,21,36]. VEGF did not show any specific pattern variation around TPs. Hence the 100% TPs positivity of peri-implantitis biopsies, lack of any sign of their specific pathological effects or expressed neovascularization in vicinity of TPs did not allow to directly corelate neovascularization pattern and VEGF expression with TPs in peri-implantitis.

Another factor that may impact the specific neovascularization patter in peri-implantitis are structural specificities of peri-implant tissues since decreased vascular supply in peri-implant tissues [54] may lead to compensatory enhanced neovascularization in inflammatory conditions. The lack of periodontal ligament affects peri-implant biology in many aspects while decline in fibroblastic mesenchymal cells directly affects immunological hemostasis and blood vessels function, contributing to development of immunopathologies and chronic inflammation [55].

The validated diagnostic markers are still lacking in implantology since majority of conducted biomarker studies are only observational and do not adhere to the guidelines for validation diagnostic studies [9,30]. Histopathological profiling is the first step in identification of disease/form-specific biological characteristics and related grading criteria in medicine, and according to pathological assessment VEGF answered the requests for candidate-marker for peri-implantitis. Moreover, based on positive correlation between tissue and soluble VEGF levels, it was confirmed that VEGF assessment in PICF represents the promising and easy-to-perform diagnostic method, since the biopsy harvesting does not represent a routine procedure in implantology.

VEGF was detectable in 85% of peri-implantitis specimens in significantly higher concentration compared to PIM and HI which is in accordance with previously reported studies [41,56,57]. VEGF was significantly correlated to all standard clinical endpoints, which also conforms previous findings [41] and confirms its diagnostic suitability in implantology [30].

VEGF convincingly reflected neovascularization and showed high specificity to peri-implantitis (96%). Moreover, VEGF was strongly correlated to all standard clinical endpoints and significantly higher compared to PIM and HI, but its respective accuracy (70%) and sensitivity in discriminating respective peri-implant conditions was modest which was particularly expressed in PIM (34%) where the sensitivity rate was about 2-fold lower than in HI (60%). Such decreased VEGF sensitivity associated with variable VEGF concentrations in PIM might be possibly explained by the fact that pattern of PIM conversion into peri-implantitis remains unclear [2] and limited capacity of clinical parameters to discriminate early peri-implantitis from PIM [9]. Moreover, distinctively increased VEGF in peri-implantitis, its 96%-specificity and identified peri-implantitis patient cluster with exceeding concentrations (VEGF>8.896 ng/mL) 8-fold higher then second cluster (<0.208 ng/mL) are strongly suggestive that VEGF may reflect peri-implantitis severity or its different biological forms. This is driven by the fact that production directly affects the intensity of local inflammation and stimulates growth of granulation tissue on count of tissue destruction [26]. MLAs have outstanding capacity to discriminate different biological forms of diseases with complex inflammatory patterns [58,59], thus the future studies should decrypt the biological background of identified peri-implantitis clusters in aid of comprehensive molecular profiling and IVD diagnostic methods.

The present study exhibits some limitations that mostly relates to the relatively small sample size and inability to quantitatively correlate Ti with immunopathological parameters and VEGF concentrations in lack of optimized markers for Ti assessment [50]. The prospective studies designed according to the guidelines for biomarker validation [30,60], in a larger sample and with appropriate patient clustering based on severity of peri-implant destruction [61] are required to establish diagnostic ranges of VEGF and its capacity to reflect disease severity. VEGF may represent promising treatment target for immunomodulatory therapy, since VEGF attenuation might diminish disease severity and decelerate disease progression [62]. Biological definition of clinical forms built on advanced molecular assessment of disease seems to be the single safe step toward successful management of peri-implant diseases thus the diagnostic studies adhering to the guidelines for biomarker validation are urgently needed in implantology. With this regard, the elucidation of the cross-talk pattern between peri-implant inflammation and neovascularization undeniably remains of vast importance for appropriate orientation of respective clinical strategies [24], thus representing the major implication for the future research in this field. Finally, the increased clinical vigilance in professional and homecare regimen strategies for dental implants in terms of preservation of the physical and chemical implant surface integrity is of paramount importance for the success of implant therapy.

5. Conclusion

Within limitations of the study, expressed neovascularization is intrinsic characteristic of peri-implantitis while VEGF may accurately reflect it, but the future research is necessary to elucidate the exact cause behind this pathological pattern for appropriate orientation of the treatment strategies in management of peri-implantitis. VEGF showed modest diagnostic capacity as biomarker of peri-implantitis.

Declaration of Competing Interest

The authors declare no conflict of interest.

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