

Meteorin-like protein, IL-1 β , TNF- α , and IL-10 levels in peri-implant crevicular fluid in peri-implant health and peri-implantitis: a comparative cross-sectional study

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Abstract

Background: Peri-implantitis is a plaque-associated inflammatory disease that leads to progressive loss of the bone supporting dental implants, yet clinical probing and radiography alone often detect it only after substantial tissue damage has already occurred. Meteorin-like (Metrnl), a recently described immunoregulatory cytokine linked to anti-inflammatory macrophage activity, has been associated with disease severity in periodontitis, but has not previously been examined in the peri-implant crevicular fluid (PICF) compartment. This study aimed to compare PICF Metrnl concentrations between peri-implant health and peri-implantitis and to explore their relationship with local pro- and anti-inflammatory cytokines and clinical parameters.

Methods: In this comparative cross-sectional study, 68 participants (34 with peri-implant health and 34 with peri-implantitis; one implant per participant) were assessed according to the 2017 World Workshop case definitions. Clinical parameters (plaque index, bleeding on probing, relative attachment level, and probing depth) were recorded by a single calibrated examiner, and PICF was collected using standardized paper strips. PICF eluate concentrations of Metrnl, IL-1 β , TNF- α , and IL-10 were measured using commercially available ELISA kits. Metrnl was expressed in ng/mL, whereas IL-1 β , TNF- α , and IL-10 were expressed in pg/mL. Group comparisons used independent-samples or Welch's t-tests and Mann–Whitney U tests as appropriate. Associations with Metrnl were evaluated using Spearman correlations calculated for the whole sample, separately within each diagnostic group, and as group-adjusted partial correlations; ROC analysis was exploratory.

Results: PICF Metrnl concentrations were markedly lower in peri-implantitis than in peri-implant health, while IL-1 β and TNF- α were higher and IL-10 was lower (all $p < 0.001$). Strong whole-sample correlations between Metrnl, cytokines, and clinical parameters were largely attributable to separation between the diagnostic groups. Within the peri-implantitis group, positive correlations remained between Metrnl and TNF- α and between Metrnl and bleeding on probing. In this selected sample, Metrnl showed complete separation between the groups in exploratory ROC analysis; this finding should not be interpreted as a validated diagnostic threshold.

Conclusions: PICF Metrnl concentrations differed between peri-implant health and peri-implantitis and varied with the local inflammatory profile in this sample. These findings identify Metrnl as a candidate biomarker for further investigation but do not establish diagnostic validity or a causal role. Independent, prospectively recruited cohorts that include intermediate peri-implant conditions are needed before clinical application can be considered.

Background

Peri-implantitis is a plaque-associated pathological condition characterized by inflammation of the peri-implant mucosa and progressive loss of supporting bone [1, 2]. A systematic review and meta-analysis estimated its prevalence at 19.5% at the patient level and 12.5% at the implant level, although estimates varied substantially according to the case definitions and diagnostic thresholds applied across

studies [3]. If left uncontrolled, peri-implantitis may require complex therapeutic interventions and ultimately jeopardize implant retention [1, 2]. Its development and progression involve a complex interaction between dysbiotic microbial biofilms and a dysregulated host immune response, resulting in sustained inflammatory signaling and osteoclast-mediated bone resorption [1, 4].

The diagnosis of peri-implantitis is primarily based on clinical signs of inflammation, including bleeding and/or suppuration on probing, an increase in probing depth relative to baseline measurements, and radiographic evidence of progressive bone loss beyond initial bone remodelling [1, 5]. Although indispensable for diagnosis and monitoring, these parameters capture different aspects of the disease: bleeding and suppuration indicate the presence of peri-implant inflammation, whereas changes in probing depth and radiographic bone levels reflect structural tissue breakdown. Their interpretation may also be influenced by probing force, accessibility around the prosthetic reconstruction, implant position, and the availability and standardization of baseline records [5, 6]. However, clinical and radiographic parameters characterize the clinical phenotype and extent of tissue destruction but provide limited information on the underlying local host-response profile [6, 7]. Consequently, molecular mediators in peri-implant biofluids have been investigated as adjunctive measures for the biological characterization of peri-implant conditions rather than as substitutes for established diagnostic criteria [7, 8].

Peri-implant crevicular fluid (PICF) is a site-associated biological fluid that can be collected from the peri-implant sulcus using absorbent paper strips and contains cytokines, enzymes, tissue-degradation products, and mediators of bone metabolism [7]. Because PICF is obtained in close proximity to the sampled implant, it has been extensively investigated as a medium for assessing the local biological environment and as a potential adjunct to clinical and radiographic evaluation [7, 8]. Among the most frequently investigated PICF biomarkers, interleukin (IL)-1 β and tumour necrosis factor alpha (TNF- α) are proinflammatory cytokines involved in inflammatory amplification and osteoclastogenic signalling, and their levels have generally been reported to be higher at peri-implantitis sites than at healthy peri-implant sites [4, 7, 9]. However, heterogeneity in case definitions, sampling procedures, analytical methods, and reporting units has prevented the establishment of clinically validated thresholds for these biomarkers [7–9]. In contrast, IL-10 is an anti-inflammatory cytokine involved in the counter-regulation of immune responses, and its assessment alongside proinflammatory mediators may provide a more comprehensive characterization of the local inflammatory profile [9].

Meteorin-like protein (Metrnl), also termed meteorin- β , is a secreted immunoregulatory protein expressed in mucosal and barrier tissues and by activated myeloid cells; it is also produced in adipose tissue and skeletal muscle [10, 11]. Metrnl production has been associated with alternatively activated, M2-like macrophages, suggesting a potential role in the regulation of innate and adaptive immune responses [10, 12]. Experimental evidence indicates that Metrnl can influence macrophage polarization and attenuate proinflammatory and osteolytic signalling, although its mechanisms of action and tissue-specific effects have not been fully elucidated [12, 13]. Collectively, these immunoregulatory and bone-related effects provide a biological rationale for investigating Metrnl in inflammatory conditions characterized by dysregulated host responses and bone loss, including peri-implantitis [4, 12, 13].

The potential relevance of Metrnl to oral inflammatory diseases has recently been investigated in periodontal tissues and oral fluids. In a comparative cross-sectional study, salivary Metrnl concentrations were significantly lower in individuals with periodontitis than in periodontally healthy individuals [14]. The findings in gingival crevicular fluid varied according to how Metrnl was expressed: its concentration was significantly lower in individuals with gingivitis and periodontitis than in periodontally healthy individuals, whereas its total amount collected over 30 seconds did not differ significantly among the groups [14]. The total amount of Metrnl in gingival crevicular fluid was also inversely correlated with the corresponding total amount of IL-1 β [14]. These findings suggest an association between Metrnl and the periodontal inflammatory environment but also demonstrate that its observed pattern may depend on the biological fluid and the metric used for biomarker quantification. Although periodontitis and peri-implantitis share core biofilm-mediated inflammatory pathways, differences in peri-implant tissue organization and inflammatory lesion characteristics limit the direct extrapolation of periodontal findings to peri-implant sites [2, 4]. To our knowledge, PICF Metrnl concentrations and their relationships with pro- and anti-inflammatory cytokines have not previously been evaluated in peri-implantitis.

The primary aim of this study was to compare PICF Metrnl concentrations between individuals with peri-implant health and those with peri-implantitis. Secondary aims were to compare PICF concentrations of IL-1 β , TNF- α , and IL-10 between the two groups. As pre-specified exploratory analyses, we investigated the correlations suppuration on probing), a probing depth ≥ 5 mm at ≥ 1 site, and radiographic evidence of peri-implant bone loss. All included implants supported fixed cement-retained restorations; no screw-retained or removable implant-supported restorations were included.

In participants with more than one eligible implant, only one implant per participant was included to avoid within-subject clustering. In the peri-implantitis group, the implant with the most pronounced disease severity — based on probing depth, bleeding on probing, and radiographic bone loss — was selected; in the peri-implant health group, the implant was selected at random when more than one qualifying implant was present.

Clinical examination

All clinical measurements were performed by a single calibrated examiner using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA) with 1-mm graduations and a 0.5-mm tip diameter. Intra-examiner reproducibility was assessed in 10 individuals not otherwise included in the study, with measurements repeated on two occasions; agreement exceeded 90%.

At each implant, six sites (mesiobuccal, mid-buccal, distobuccal, mesiolingual/palatal, mid-lingual/palatal, and distolingual/palatal) were recorded for the following parameters:

- *Plaque index (PI)*, scored 0–3 [15], with the mean of the six site scores used as the implant-level value.

- *Probing depth (PD)*, measured from the mucosal margin to the base of the peri-implant sulcus/pocket using a light, standardized force (≤ 0.25 N), with the probe kept as parallel as possible to the long axis of the implant [16]; the implant-level PD was the mean of the six sites.
- *Relative attachment level (RAL)*, measured from the fixed margin of the implant-supported restoration to the base of the sulcus/pocket using the same probing technique; the implant-level RAL was the mean of the six sites.
- *Bleeding on probing (BOP)*, recorded as present/absent within 10 seconds of probing [17], and expressed per implant as the percentage of bleeding sites out of the six examined.

between Metrnl and each of these cytokines and evaluated the ability of PICF Metrnl concentrations to discriminate peri-implantitis from peri-implant health using receiver operating characteristic (ROC) curve analysis. We hypothesized that PICF Metrnl concentrations would differ between peri-implant health and peri-implantitis and would be correlated with the local pro- and anti-inflammatory cytokine profile.

Methods

This comparative cross-sectional study was conducted at the Department of Periodontology, Faculty of Dentistry, Atatürk University, Erzurum, Turkey. The study was approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki; written informed consent was obtained from all participants prior to enrolment.

Participants

Individuals presenting to the Department of Periodontology with peri-implant complaints or for routine periodontal/peri-implant review, and who had at least one dental implant, were screened for eligibility. Inclusion criteria were age >18 years, absence of systemic disease, and presence of at least one implant supporting a fixed, cement-retained prosthetic restoration that had been in function for at least 1 year and met the eligibility criteria for either peri-implant health or peri-implantitis. Individuals were excluded if they were pregnant or lactating; current smokers; had used antibiotics or corticosteroids within the preceding 6 months; had an implant-supported removable prosthesis; or had received periodontal or peri-implant treatment within the preceding 6 months.

Individuals were excluded if they had systemic disease; were pregnant or lactating; were current smokers; had used antibiotics or corticosteroids within the preceding 6 months; had a screw-retained or removable implant-supported prosthesis; had an implant in function for less than 1 year; had declined participation; or had received periodontal or peri-implant treatment within the preceding 6 months.

Of the 85 individuals screened, 17 were excluded before enrolment because eligibility criteria were not met or participation was declined. The recorded reasons included systemic disease, screw-retained or removable restorations, implants in function for less than 1 year, and declining participation. The remaining 68 participants were included in the study, comprising 34 participants with peri-implant health and 34 with peri-implantitis. No missing data were present among the included participants.

Sample size calculation

The required sample size was calculated a priori using G*Power version 3.1.9.7 for a two-tailed independent-samples comparison between two groups. The calculation was based on an anticipated standardized effect size of Cohen's $d = 0.70$, an α level of 0.05, a statistical power of 80%, and a 1:1 allocation ratio. The minimum required sample size was 68 participants, corresponding to 34 participants per group. Accordingly, 34 participants with peri-implant health and 34 participants with peri-implantitis were included, with one implant evaluated per participant.

Study groups and implant selection

Participants underwent clinical and radiographic evaluation and were classified according to the case definitions of peri-implant health and peri-implantitis established by the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [1, 5]. The peri-implant health group comprised 34 implants with no clinical signs of inflammation (oedema, erythema, suppuration, or bleeding on probing) and no radiographic bone loss beyond initial physiological remodelling. The peri-implantitis group comprised 34 implants with clinical signs of inflammation (oedema, erythema, bleeding and/or

Peri-implant crevicular fluid (PICF) sampling

To minimize the effect of probing-induced disruption of the peri-implant soft-tissue seal and the risk of blood contamination on biomarker measurements, PICF sampling was performed 24 hours after completion of all clinical periodontal and peri-implant assessments, consistent with previous PICF biomarker studies [18]. The selected implant site was isolated with cotton rolls and gently air-dried. PICF was collected using sterile standardized paper strips (Periopaper™, Oraflow Inc., Plainview, NY, USA) inserted 1–2 mm into the peri-implant sulcus and left in place for 30 seconds [19]. Two strips were collected from each selected implant and pooled for biochemical analysis. Strips visibly contaminated with blood or saliva were discarded and sampling was repeated using new strips. The two strips were placed together in an Eppendorf tube containing 300 μL of phosphate-buffered saline and stored at -80°C until biochemical analysis.

Biochemical analysis

Biochemical analysis

All laboratory analyses were performed at the Department of Medical Biochemistry, Faculty of Medicine, Atatürk University. Frozen PICF samples were thawed sequentially at -20°C and $+4^\circ\text{C}$ before being brought to room temperature. The tubes were then vortexed and centrifuged at 4,500 rpm for 5 min, and the resulting supernatants were used for biochemical analysis. All assays were completed on the day of thawing.

PICF eluate concentrations of Metrnl, IL-1 β , TNF- α , and IL-10 were quantified using commercially available human sandwich enzyme-linked immunosorbent assay kits (Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China), according to the manufacturer's instructions.

Metrnl was measured using a Human Meteorin-like protein (METRNL) ELISA Kit (catalogue no. 201-12-9252), with a reported measurement range of 0.05–15 ng/mL and an analytical sensitivity of 0.042 ng/mL. IL-1 β was measured using a Human IL-1 β ELISA Kit (catalogue no. 201-12-0144), with a reported measurement range of 20–8,000 pg/mL and an analytical sensitivity of 15.013 pg/mL. TNF- α was measured using a Human TNF- α ELISA Kit (catalogue no. 201-12-0083), with a reported measurement range of 3–900 ng/L and an analytical sensitivity of 2.827 ng/L. Because 1 ng/L is numerically equivalent to 1 pg/mL, TNF- α results were converted to and reported in pg/mL. IL-10 was measured using a Human IL-10 ELISA Kit (catalogue no. 201-12-0103), with a reported measurement range of 10–3,000 pg/mL and an analytical sensitivity of 9.012 pg/mL. For all four assays, the manufacturer-reported intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively.

Briefly, samples and analyte-specific standards were added to antibody-precoated 96-well microplates. After incubation and washing, biotin-conjugated detection antibodies and streptavidin-conjugated horseradish peroxidase were added sequentially. Following further washing, chromogenic reagents were added, and the reactions were terminated using the supplied acidic stop solution. Absorbance was measured at 450 nm using a microplate spectrophotometer. Analyte concentrations were calculated from the corresponding analyte-specific standard curves. Metrnl concentrations were expressed in ng/mL, whereas IL-1 β , TNF- α , and IL-10 concentrations were expressed in pg/mL.

Statistical analysis

Primary statistical analyses were performed using MedCalc Statistical Software version 20.011 (MedCalc Software Ltd, Ostend, Belgium). Additional multivariable and rank-based sensitivity analyses and false-discovery-rate calculations were performed using Python version 3.12.13 with NumPy version 2.3.5 and SciPy version 1.17.0.

The distribution of each continuous variable was assessed separately within each diagnostic group using the Shapiro–Wilk test. Variables consistent with a normal distribution in both groups were compared using the independent-samples t-test. Welch's t-test was used when the assumption of homogeneity of variances, assessed using Levene's test, was not met. Variables that deviated from normality in either group were compared using the Mann–Whitney U test. Sex distributions were compared using Pearson's chi-square test. For the biomarker comparisons, effect sizes were reported as Hedges' g for t-test comparisons and absolute rank-biserial correlation ($|r_{rb}|$) for Mann–Whitney U comparisons. The clinical characteristics used directly or indirectly in diagnostic-group definition or implant selection were presented descriptively, without effect-size interpretation.

Associations between Metrnl concentration and IL-1 β , TNF- α , IL-10, plaque index, BOP, RAL, PD, and age were evaluated using Spearman's rank correlation coefficient (ρ). Correlations were calculated for the

whole sample and separately within each diagnostic group. Group-adjusted partial Spearman correlations were obtained by ranking both variables, residualizing their ranks against binary diagnostic-group membership, and correlating the resulting residuals. Whole-sample and group-adjusted estimates were interpreted together because pooling two diagnostically distinct groups may produce correlations primarily reflecting between-group separation. These correlation analyses were considered exploratory.

As a sensitivity analysis, the between-group difference in Metrnl concentration was further examined using a multivariable linear regression model with Metrnl concentration as the dependent variable and diagnostic group, age, and plaque index as explanatory variables. Heteroscedasticity-consistent HC3 standard errors were used to reduce sensitivity to unequal residual variances. A rank-based regression analysis including the same explanatory variables was additionally performed to assess the robustness of the group association to distributional assumptions. Because plaque index was closely associated with diagnostic-group membership, the adjusted estimates were interpreted as sensitivity analyses and not as causal effects.

The ability of PICF Metrnl concentration to distinguish peri-implantitis from peri-implant health was examined using exploratory receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) and its 95% confidence interval were calculated. Complete separation was observed between the Metrnl distributions of the two groups; consequently, every threshold between the highest value in the peri-implantitis group and the lowest value in the peri-implant health group produced identical classification in this dataset. The midpoint of this interval was therefore reported as a descriptive, sample-derived threshold rather than as a uniquely Youden-optimized or clinically validated cut-off. Sensitivity and specificity were reported with exact Clopper–Pearson 95% confidence intervals.

All tests were two-tailed. The primary between-group comparison of Metrnl concentration was evaluated at an α level of 0.05. Nominal p values were reported for the exploratory correlation analyses. To evaluate the potential influence of multiple testing, Benjamini–Hochberg false-discovery-rate correction was additionally applied across all computable Metrnl correlation tests within each analysis stratum. An FDR-adjusted q value <0.05 was considered statistically significant in this sensitivity analysis.

Results

Demographic and clinical characteristics

During the screening period, 85 individuals were assessed for eligibility. Seventeen individuals were excluded before enrolment because eligibility criteria were not met or participation was declined. The recorded reasons included systemic disease, screw-retained or removable restorations, implants in function for less than 1 year, and declining participation. The remaining 68 participants were included in the study, comprising 34 participants with peri-implant health and 34 with peri-implantitis. No missing values were present for the variables included in the analyses.

A total of 68 participants, each contributing one implant, were included: 34 with peri-implant health and 34 with peri-implantitis. No statistically significant between-group differences were observed in sex distribution or age (both $p > 0.05$). Plaque index, BOP, RAL, and PD were higher in the peri-implantitis group (all $p < 0.001$; Table 1). However, differences in BOP, PD, and RAL were interpreted descriptively because these clinical characteristics were directly or indirectly related to the diagnostic-group definitions and implant-selection criteria.

Table 1. Demographic and clinical characteristics of the study groups

Variable	Peri-implant health (n = 34)	Peri-implantitis (n = 34)	Test statistic	p
Sex, female/male, n (%)	14/20 (41.2/58.8)	15/19 (44.1/55.9)	$\chi^2 = 0.060$	0.806
Age, years	49.1 $\pm$ 9.3	52.8 $\pm$ 8.9	$t = -1.665$	0.101
Plaque index	0.67 (0.54–0.83)	1.33 (1.17–1.67)	$U = 43.5$	<0.001
BOP, %	0 (0–0)	66.67 (50.00–83.33)	$U = 0.0$	<0.001
RAL, mm	3.22 $\pm$ 0.51	7.25 $\pm$ 0.83	$t = -24.250$	<0.001
PD, mm	3.22 $\pm$ 0.38	7.02 $\pm$ 0.62	$t = -30.413$	<0.001

Data are presented as mean $\pm$ standard deviation or median (Q1–Q3), according to their distribution. Sex is presented as number and percentage. Clinical differences in BOP, PD, and RAL should be interpreted descriptively because these characteristics were directly or indirectly related to the definition and selection of the diagnostic groups. $\square$ Welch's t -test was used because the assumption of equal variances was not met. BOP, bleeding on probing; RAL, relative attachment level; PD, probing depth.

PICF biomarker levels

PICF concentrations of the pro-inflammatory cytokines IL-1 β and TNF- α were markedly higher in the peri-implantitis group, whereas the anti-inflammatory cytokine IL-10 and Metrnl were markedly lower, compared with the peri-implant health group (all $p < 0.001$; Table 2). Notably, the magnitude of separation between groups was substantial for all four biomarkers, with minimal or no overlap in observed value ranges.

Table 2. Comparison of PICF biomarker levels between the study groups

Biomarker	Peri-implant health (n = 34)	Peri-implantitis (n = 34)	Test statistic	p	Effect size
IL-1 β , pg/mL	89.81 (85.71–103.18)	235.24 (223.63–251.18)	U = 2.0	<0.001	r _{rb} = 0.997
TNF- α , pg/mL	107.6 $\pm$ 12.6	289.4 $\pm$ 13.9	t = -56.430	<0.001	g = 13.530
IL-10, pg/mL	901.61 (832.91–948.26)	553.42 (505.47–589.67)	U = 1156.0	<0.001	r _{rb} = 1.000
MetrnI, ng/mL	7.84 (7.64–8.10)	4.29 (3.99–4.45)	U = 1156.0	<0.001	r _{rb} = 1.000

Data are mean $\pm$ SD or median (Q1–Q3), according to distribution (see Supplementary Table S1).

Correlations between MetrnI and cytokine/clinical parameters

In the whole sample, MetrnI correlated inversely with IL-1 β (ρ = -0.776), TNF- α (ρ = -0.663), plaque index (ρ = -0.701), BOP (ρ = -0.723), RAL (ρ = -0.726), and PD (ρ = -0.724), and positively with IL-10 (ρ = 0.728; all p < 0.001); age was not significantly correlated with MetrnI (ρ = -0.140, p = 0.257) (Tables 3 and 4). Most associations were attenuated within the diagnostic groups and after adjustment for group membership, suggesting that the whole-sample coefficients largely reflected between-group separation. Within the peri-implantitis group, MetrnI remained positively correlated with TNF- α (ρ = 0.511, p = 0.002; FDR q = 0.008) and BOP (ρ = 0.630, p < 0.001; FDR q < 0.001). Group-adjusted correlations were also positive for TNF- α (ρ = 0.348, p = 0.004; FDR q = 0.014) and BOP (ρ = 0.446, p < 0.001; FDR q = 0.001). The BOP–MetrnI correlation could not be calculated within the peri-implant health group because BOP was uniformly 0%. These exploratory associations should not be interpreted as evidence of causality or effect modification.

In the sensitivity analysis adjusted for age and plaque index, peri-implantitis remained associated with a lower MetrnI concentration (adjusted β = -3.71 ng/mL, 95% CI -3.98 to -3.44; p < 0.001). Neither age (β = 0.002 ng/mL per year, 95% CI -0.006 to 0.010; p = 0.622) nor plaque index (β = 0.068 ng/mL per unit, 95% CI -0.175 to 0.311; p = 0.582) showed an independent association with MetrnI within this model. The group association was also retained in the rank-based sensitivity analysis (p < 0.001). Because plaque index was closely associated with diagnostic-group membership, these adjusted findings should be interpreted as sensitivity analyses rather than causal estimates.

Table 3. Spearman correlations between PICF MetrnI and cytokine concentrations

Cytokine	Whole sample ρ (p)	Health group ρ (p)	Peri-implantitis group ρ (p)	Group-adjusted ρ (p)
IL-1 β , pg/mL	-0.776 (<0.001)	-0.180 (0.309)	-0.038 (0.831)	-0.112 (0.364)
TNF- α , pg/mL	-0.663 (<0.001)	0.185 (0.295)	0.511 (0.002)	0.348 (0.004)
IL-10, pg/mL	0.728 (<0.001)	0.016 (0.927)	-0.195 (0.269)	-0.089 (0.468)

Group-adjusted values are partial Spearman correlations controlling for diagnostic-group membership. Nominal p values are presented in the table; the accompanying text reports the Benjamini–Hochberg FDR sensitivity analysis across the eight correlations within each analysis stratum.

Table 4. Spearman correlations between PICF Metrnl and clinical parameters

Clinical variable	Whole sample ρ (p)	Health group ρ (p)	Peri-implantitis group ρ (p)	Group-adjusted ρ (p)
Plaque index	-0.701 (<0.001)	-0.092 (0.605)	0.073 (0.682)	-0.004 (0.974)
BOP, %	-0.723 (<0.001)	Not computable (zero variance)	0.630 (<0.001)	0.446 (<0.001)
RAL, mm	-0.726 (<0.001)	0.273 (0.119)	-0.067 (0.706)	0.102 (0.407)
PD, mm	-0.724 (<0.001)	0.241 (0.169)	-0.016 (0.929)	0.112 (0.362)
Age, years	-0.140 (0.257)	-0.091 (0.609)	0.174 (0.325)	0.035 (0.779)

Group-adjusted values are partial Spearman correlations controlling for diagnostic-group membership. BOP was uniformly 0% in the peri-implant health group; therefore, the within-group BOP–Metrnl correlation was not computable. Nominal p values are presented in the table; the accompanying text reports the Benjamini–Hochberg FDR sensitivity analysis.

Diagnostic performance of Metrnl

Exploratory ROC analysis yielded an AUC of 1.000 (95% CI 1.000–1.000) in this selected sample, reflecting complete separation between the observed Metrnl ranges in peri-implantitis (2.78–4.83 ng/mL) and peri-implant health (7.40–8.33 ng/mL) (Table 5)(Figure 3). Any threshold between 4.83 and 7.40 ng/mL produced identical classification. The midpoint of this interval (≤ 6.11 ng/mL) is presented descriptively and yielded an apparent sensitivity of 100% (95% CI 89.7–100%) and specificity of 100% (95% CI 89.7–100%). These in-sample estimates may be affected by spectrum and optimism bias and should not be interpreted as an externally validated diagnostic threshold.

Table 5. Diagnostic performance of PICF Metrnl

Parameter	Value	95% CI / range
AUC	1.000	1.000–1.000
Descriptive midpoint	≤6.11 ng/mL	4.83–7.40 ng/mL
Sensitivity	100%	89.7–100%
Specificity	100%	89.7–100%
Observed range (peri-implantitis / health)	2.78–4.83 / 7.40–8.33 ng/mL	—

AUC, area under the curve. Interval within which every threshold yielded identical classification in this sample; 6.11 ng/mL represents the midpoint of this interval and is not a validated clinical cut-off. Exact Clopper–Pearson 95% CI. Estimates are uncorrected for in-sample optimism.

Discussion

This study evaluated Metrnl concentrations in standardized peri-implant crevicular fluid (PICF) eluates, together with the pro-inflammatory cytokines IL-1 β and TNF- α and the anti-inflammatory cytokine IL-10, in individuals with peri-implant health and peri-implantitis. Metrnl concentrations were lower in the peri-implantitis group, whereas IL-1 β and TNF- α concentrations were higher and IL-10 concentrations were lower. Although Metrnl was strongly correlated with several cytokine and clinical parameters in the whole sample, most of these associations were substantially attenuated within the diagnostic groups and after adjustment for group membership. This pattern suggests that the whole-sample correlations predominantly reflected the marked separation between the selected clinical phenotypes rather than uniform relationships operating within each group. We are not aware of a previous study evaluating Metrnl specifically in PICF; therefore, the present findings should be regarded as preliminary and hypothesis-generating.

The higher IL-1 β and TNF- α concentrations observed in peri-implantitis are broadly consistent with the inflammatory profile described in the PICF literature. Both cytokines participate in the amplification of local inflammatory responses and may contribute to osteoclastogenic signalling and peri-implant tissue destruction [4, 7, 9, 20]. Nevertheless, the magnitude and direction of biomarker differences reported across studies are heterogeneous, reflecting variation in case definitions, disease severity, sampling time, number of sampled sites, collection and elution procedures, analytical platforms, and whether results are expressed as concentration or total amount [7–9, 20]. Accordingly, the absolute cytokine concentrations observed in the present study should be interpreted in relation to the specific sampling and analytical protocol rather than directly compared across studies using different matrices or reporting metrics.

IL-10 concentrations were lower in peri-implantitis than in peri-implant health. As an anti-inflammatory cytokine, IL-10 may counter-regulate pro-inflammatory signalling and contribute to the control of tissue-destructive immune responses. However, previous studies have reported inconsistent patterns of IL-10 in periodontal and peri-implant biofluids, including increased, decreased, or unchanged levels in diseased conditions [9, 20]. These inconsistencies may reflect differences in disease stage, local cellular composition, sampling methodology, fluid volume, and the balance between cytokine production, consumption, and degradation. Therefore, the lower IL-10 concentration observed in the present peri-implantitis group may indicate an altered local regulatory environment but should not be interpreted as direct evidence of impaired IL-10 production or activity.

The direction of the Metrnl difference is consistent with the available oral-fluid evidence. Saglam et al. reported lower salivary Metrnl concentrations in individuals with periodontitis than in periodontally healthy individuals and identified inverse associations between salivary Metrnl and periodontal clinical parameters [14]. In gingival crevicular fluid, however, their findings varied according to the reporting metric: Metrnl concentration differed among the periodontal groups, whereas the total amount collected over 30 seconds did not [14]. This distinction is relevant to the interpretation of the present results. PICF volume was not quantified in our study; consequently, total Metrnl and cytokine amounts per sampled implant could not be calculated. The present findings therefore represent analyte concentrations in standardized fixed-volume eluates prepared from two pooled paper strips. Direct comparison with studies reporting site-level total amounts should accordingly be undertaken with caution.

The substantially greater apparent discrimination observed for PICF Metrnl in the present study than that reported for salivary Metrnl by Saglam et al. may have several explanations [14]. PICF is sampled in close proximity to the implant and may more directly reflect the local peri-implant environment, whereas saliva represents a composite fluid influenced by multiple oral and systemic sources [7, 8]. However, differences in biofluid compartment alone are unlikely to explain the complete separation observed here. The study compared strictly healthy implants with clinically established peri-implantitis, did not include peri-implant mucositis or intermediate disease presentations, and selected the most severely affected implant when more than one diseased implant was eligible. These design features may have magnified the biological contrast between groups and increased the apparent diagnostic performance of Metrnl.

Metrnl has been described as an immunoregulatory protein associated with barrier tissues, activated myeloid cells, and alternatively activated M2-like macrophages [10, 12, 21]. Experimental evidence indicates that Metrnl may influence macrophage polarization and attenuate pro-inflammatory and osteolytic signalling, providing a biologically plausible framework for its lower concentration in peri-implantitis [12, 13]. The concurrent reduction in IL-10 and elevation of IL-1 β and TNF- α are compatible with an altered balance between regulatory and pro-inflammatory pathways. However, the cross-sectional nature of the present study does not establish whether lower Metrnl contributes to peri-implant inflammation, results from the inflammatory environment, or reflects changes in the cellular composition of diseased peri-implant tissues.

The correlation findings further suggest that the relationship between Metrnl and inflammation may be more complex than a simple anti-inflammatory antagonism. In the whole sample, Metrnl was inversely correlated with IL-1 β , TNF- α , plaque index, BOP, RAL, and PD and positively correlated with IL-10. Most of these relationships were no longer evident within the individual diagnostic groups or after adjustment for group membership, suggesting that they largely arose from the contrast between health and peri-implantitis. Conversely, Metrnl was positively correlated with TNF- α and BOP within the peri-implantitis group, and these associations remained evident after false-discovery-rate adjustment. These findings may indicate that, within an already inflamed peri-implant environment, Metrnl co-varies with aspects of local inflammatory activity rather than uniformly opposing them. Similar context-dependent patterns have been reported in systemic inflammatory conditions, including psoriatic arthritis and rheumatoid arthritis, in which increased Metrnl has been associated with inflammatory activity [22, 23]. Nevertheless, the present exploratory correlations do not establish a mechanistic interaction between Metrnl, TNF- α , and BOP.

Adjustment for age and plaque index in the sensitivity analysis did not materially alter the association between diagnostic group and Metrnl concentration. This finding suggests that the observed group difference was not explained solely by the measured differences in age or plaque accumulation. However, plaque index was closely related to diagnostic-group membership, and residual confounding by unmeasured implant-, prosthesis-, maintenance-, and patient-related factors remains possible. The adjusted model should therefore be interpreted as a sensitivity analysis rather than as evidence of an independent or causal effect of peri-implantitis on Metrnl.

Exploratory ROC analysis demonstrated complete separation of Metrnl concentrations between the two diagnostic groups in this sample. Although this resulted in an AUC of 1.000 and apparent sensitivity and specificity of 100%, these estimates should not be interpreted as evidence of perfect diagnostic accuracy. Any threshold located between the highest peri-implantitis value and the lowest peri-implant health value produced identical in-sample classification. The reported midpoint therefore represents a descriptive, sample-derived value rather than a uniquely optimized or clinically validated threshold. The exclusion of peri-implant mucositis and other intermediate presentations, the use of strictly healthy comparators, and the absence of internal resampling or external validation may have introduced spectrum and optimism bias. Metrnl should consequently be regarded as a candidate marker of peri-implant inflammatory status rather than a stand-alone diagnostic test.

The findings nevertheless support continued investigation of PICF biomarkers as potential adjuncts to established peri-implant assessment. Clinical probing and radiographic examination remain indispensable for diagnosing and monitoring peri-implant diseases, while PICF analysis may provide complementary information regarding the local host-response profile [5, 7, 8, 20]. Future studies should determine whether Metrnl provides information beyond routinely available clinical parameters and established inflammatory biomarkers. Such studies should include peri-implant mucositis and a broader spectrum of disease severity, assess both concentration and total amount when fluid-volume measurement is available, and evaluate diagnostic models in independent populations.

The study has several strengths, including a standardized PICF collection and fixed-volume elution protocol, sampling timed to reduce probing-induced contamination, clinical measurements performed by a single calibrated examiner, inclusion of one implant per participant to avoid within-subject clustering, and concurrent assessment of Metrnl with pro- and anti-inflammatory cytokines. The separate presentation of whole-sample, within-group, and group-adjusted correlations also enabled the influence of diagnostic-group separation on the observed associations to be examined more transparently.

Several limitations should also be considered. First, the cross-sectional design precludes causal and temporal inferences and does not allow changes in biomarker concentrations during disease progression or following treatment to be evaluated. Second, the study was conducted at a single centre in a selected Turkish adult population and compared two contrasting clinical phenotypes without including peri-implant mucositis or intermediate disease presentations. Third, selecting the most severely affected implant in participants with multiple diseased implants may have increased phenotypic separation and inflated the observed effect sizes and apparent ROC performance. Fourth, PICF volume was not quantified, precluding calculation of total biomarker amounts per sampled implant and normalization to the original PICF volume. Fifth, although all implants had been in function for at least 1 year, the exact duration of function was not available for patient-level adjustment. Information regarding implant system, surface characteristics, anatomical position, maintenance history, previous periodontitis, and former smoking was not comprehensively incorporated into the analysis, leaving the possibility of residual confounding. Finally, all included implants supported cement-retained fixed restorations; therefore, the findings may not be directly generalizable to screw-retained or removable implant-supported prostheses. Larger, independently recruited and preferably multicentre studies using analytically validated assays and encompassing the full peri-implant disease spectrum are required to confirm these findings.

Conclusions

Within the limitations of this cross-sectional study, PICF Metrnl concentrations were markedly lower in peri-implantitis than in peri-implant health and were inversely associated, at the whole-sample level, with pro-inflammatory cytokines and clinical parameters of peri-implant inflammation and tissue destruction. These findings support further investigation of Metrnl as a candidate biomarker of peri-implant inflammatory status. Longitudinal and multicenter studies are needed to confirm these findings and to determine whether PICF Metrnl has practical value as an adjunct to clinical and radiographic diagnosis of peri-implant disease.

Declarations

Ethics approval and consent to participate

This study was approved by the Atatürk University Non-Interventional Clinical Research Ethics Committee (decision no. 758, 27 December 2024) and conducted in accordance with the Declaration of

Helsinki. Written informed consent was obtained from all participants prior to enrolment. The study was registered on ClinicalTrials.gov (NCT07697729).

Consent for publication

Not applicable.

Availability of data and materials

The de-identified dataset generated and analysed during the current study is available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

PET contributed to the conceptualization and methodology of the study, project administration, interpretation of the findings, and preparation of the original manuscript draft. RO contributed to the conceptualization and methodology of the study, supervised the thesis project, and critically reviewed and edited the manuscript. MAT contributed to participant recruitment, clinical sample collection, data curation, formal statistical analysis, visualization of the results, and critical revision of the manuscript. EL contributed to the biochemical methodology, laboratory analyses, and interpretation of the biochemical findings and critically reviewed the manuscript. All authors read and approved the final manuscript and agreed to be accountable for the work.

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Figures

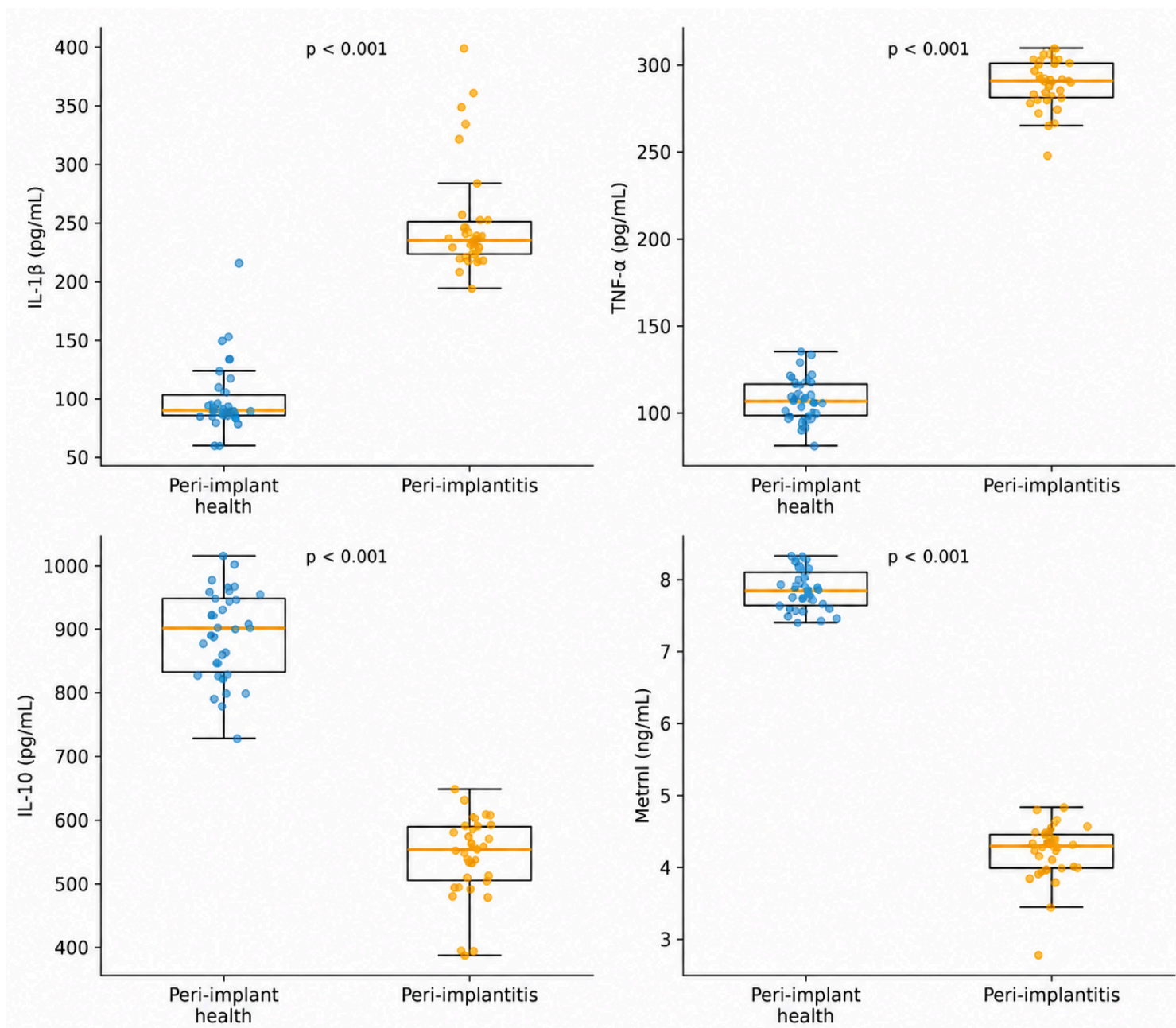
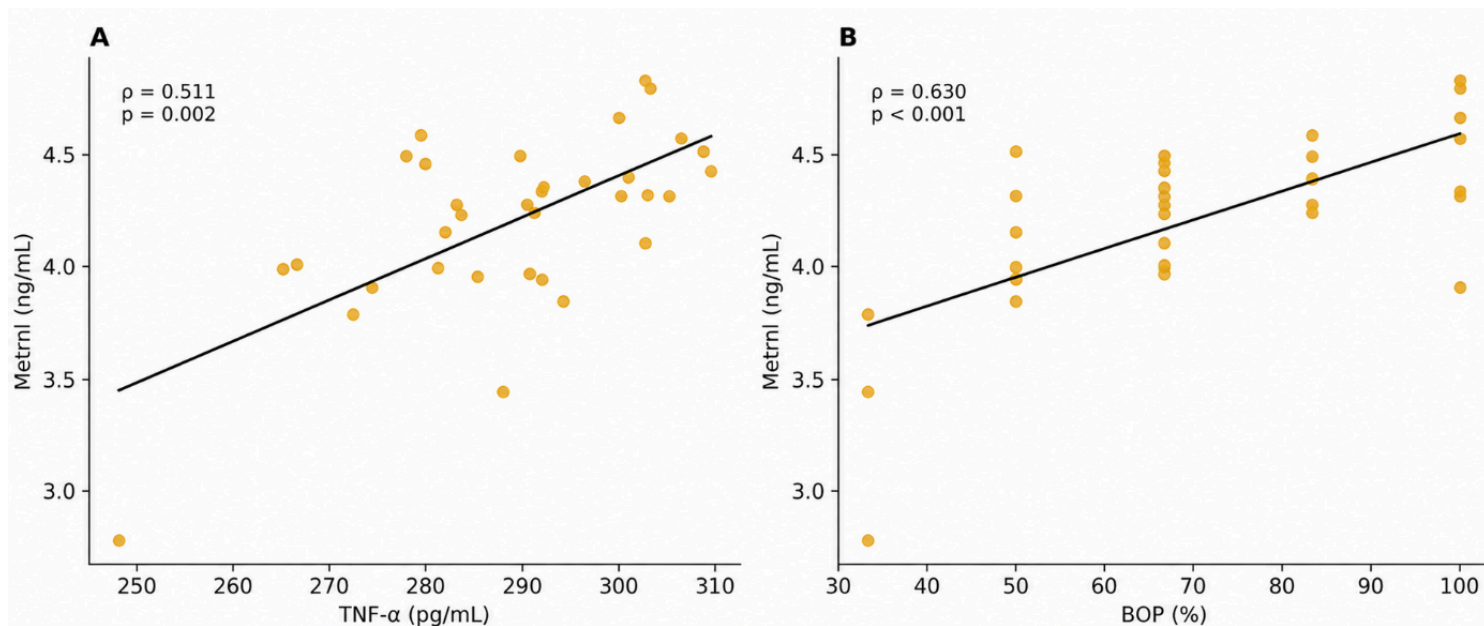


Figure 1

PICF biomarker concentrations in peri-implant health and peri-implantitis

Figure 1. PICF eluate concentrations of IL-1 β , TNF- α , IL-10, and Metrnl in the peri-implant health and peri-implantitis groups. IL-1 β , TNF- α , and IL-10 are presented in pg/mL, whereas Metrnl is presented in ng/mL. Boxes show the median and interquartile range, and individual points represent individual participants.



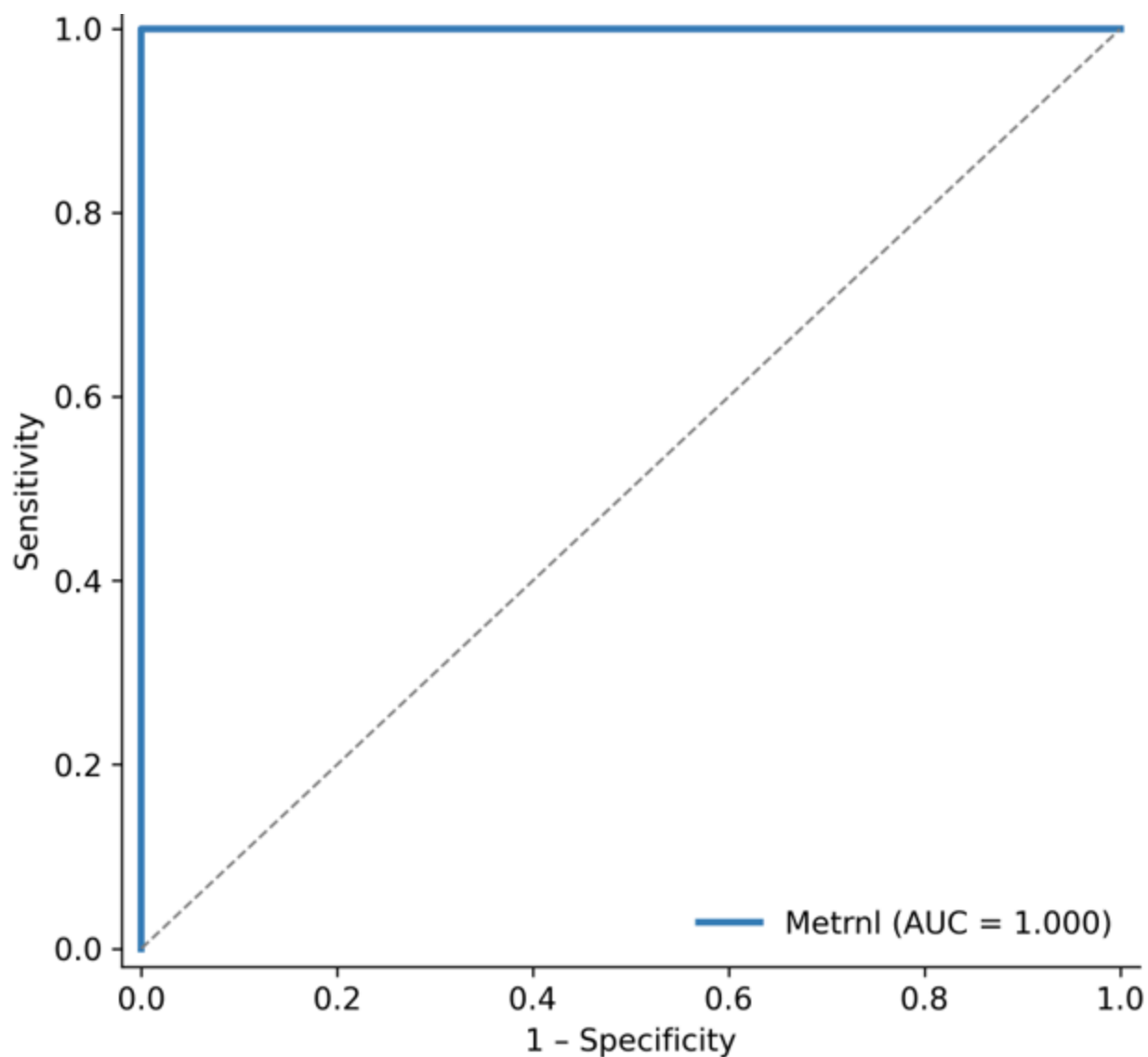


Figure 3

Receiver operating characteristic (ROC) curve for PICF Metrnl separating peri-implantitis from peri-implant health in the study sample (AUC = 1.000).

Supplementary Files

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