

STATISTICAL ANALYSIS PLAN

Association of Metrnl Expression With IL-1 β and TNF- α in Patients With Peri-Implantitis: Potential as a Novel Biomarker

ClinicalTrials.gov Identifier: NCT07697729

Organization's Unique Protocol ID: 758

Document Date: August 02, 2026

Sponsor: Atatürk University

Principal Investigator: Pınar Eser Tuna, DDS (PhD Student)

1. Study Design Summary

Observational, case-control study with a cross-sectional time perspective, comparing peri-implant health (n = 34) and peri-implantitis (n = 34) groups, total 68 implant sites. One implant site was sampled per participant.

2. Sample Size Justification

Sample size determination was based on the study reported by Sağlam et al., which found an effect size of approximately Cohen's $d = 0.50$, interpreted as a medium effect. Based on comparison of the mean difference between two independent groups, a power analysis conducted using G*Power 3.1.9.7 ($\alpha = 0.05$, power $[1-\beta] = 0.80$, two-tailed test) indicated that a minimum of 32 sample units per group was required. The studies by Guarnieri et al. and Gündoğar & Uzunkaya, which evaluated PICF biomarker levels comparing peri-implant health and peri-implantitis, were considered as supportive methodological literature for the study design (Guarnieri, Reda, et al., 2024; Gündoğar & Uzunkaya, 2021). A total of 68 implant sites were enrolled (34 per group), exceeding the minimum requirement.

3. Statistical Software

Statistical analyses were performed using MedCalc (version 20.011; MedCalc Software, Ostend, Belgium).

4. Analysis of Continuous and Categorical Variables

The normality of distribution for each continuous variable was assessed separately within each group using the Shapiro–Wilk test.

- Variables with a normal distribution in both groups were compared using the independent-samples t-test, or Welch's t-test when the assumption of homogeneity of variances (assessed by Levene's test) was violated.
- Variables that deviated from a normal distribution in either group were compared using the Mann–Whitney U test.
- The categorical variable of sex was compared between groups using Pearson's chi-square test.

Effect sizes were calculated as Hedges' g for t-test comparisons, the rank-biserial correlation (r_{rb}) for Mann–Whitney U comparisons, and Cramér's V for the chi-square comparison.

5. Correlation Analysis

Associations between PICF MetrnI concentrations and the pro- and anti-inflammatory cytokines (IL-1 β , TNF- α , IL-10) and clinical parameters (PI, BOP, CAL, PD, age) were assessed using Spearman's rank correlation coefficient (ρ), calculated for the whole sample, separately within each group, and as a partial correlation adjusting for group membership.

6. Diagnostic Performance (ROC) Analysis

The discriminative ability of PICF MetrnI concentrations for peri-implantitis versus peri-implant health was evaluated by receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), the optimal cut-off value (determined by the Youden index), and the corresponding sensitivity and specificity (with exact Clopper–Pearson 95% confidence intervals) were calculated.

7. Significance Threshold

A two-tailed p-value < 0.05 was considered statistically significant for all analyses.

8. Outcome-to-Analysis Mapping

- Primary outcome (Metrnl concentration, group comparison): independent-samples t-test / Welch's t-test / Mann–Whitney U test, as applicable (Section 4).
- Secondary outcomes (IL-1 β , TNF- α , IL-10 concentrations, group comparison): independent-samples t-test / Welch's t-test / Mann–Whitney U test, as applicable (Section 4).
- Pre-specified correlations (Metrnl vs. IL-1 β , TNF- α , IL-10): Spearman's ρ (Section 5).
- Pre-specified diagnostic performance (Metrnl AUC): ROC curve analysis (Section 6).