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# Diagnostic accuracy of bone-related biomarkers on peri-implantitis: potential of A-proliferation-inducing ligand

Alejandra Chaparro[1,3*], Valeria Ramírez[2], María José Bendek[1,3], José Nart[4], Alberto Monje[4], Andrés Pascual[4], Carolina Rojas[1,3], María Luisa Mizgier[1,3], Daniela Albers[1,2] and Víctor Beltrán[5]

## Abstract

**Objective** To study the biomarker profile in peri-implant crevicular fluid (PICF) of A proliferation-inducing ligand (APRIL), receptor activator of nuclear factor κβ (RANKL) and interleukin (IL)-23, in healthy, periimplant mucositis and peri-implantitis sites, and to explore their diagnostic accuracy on periimplantitis (PI) diagnosis.

**Materials and methods** An exploratory cross-sectional study was conducted. Sociodemographic and clinical were recorded. Implant diagnosis was made based on the latest classification consensus. PICF samples were collected with paper strips from healthy, mucositis and PI implants. The biomarkers were analyzed by Luminex assay. The diagnostic accuracy was determined through sensitivity, specificity, predictive values (PV), and receiver operating characteristic (ROC) curves.

**Results** Overall, 54 patients were recruited; 17 were healthy implants, 19 with mucositis and 18 have PI. RANKL and APRIL levels in PICF were significantly increased in PI implants compared to healthy implants ($p < 0.001$ and $p = 0.005$). IL-23 did not present differences between groups ($p > 0.05$). Positive correlations between PICF-RANKL levels and clinical attachment loss, plaque index score and bleeding on probing were observed (rho = 0.33; rho = 0.35; rho = 0.33; $p < 0.05$, respectively). Additionally, PICF-IL-23 and APRIL were correlated with the plaque index score and peri-implant probing depth (rho = 0.28; rho = 0.28, $p < 0.05$). PICF-APRIL concentrations and plaque index score were associated with PI (OR:3.01; 95%CI [1.08–8.38], $p = 0.035$, and OR:11.24; 95% CI [2.63–48.16], $p = 0.001$, respectively). The regression model, which included PICF-APRIL and plaque index, showed an AUC-ROC of 0.95, a sensitivity of 94.4%, 83.3% specificity, a positive PV of 73.9%, and a negative PV of 96.8%.

**Conclusions** Implants with PI have higher levels of APRIL and RANKL in PICF compared to healthy implants. The model that includes the levels of APRIL in PICF combined with the plaque index score leads to an enhanced accuracy of PI diagnosis. **Clinical relevance**: Clinical diagnosis of peri-implant disease can be improved with molecular tools, in this case, APRIL demonstrated high accuracy for the diagnosis of PI.

**Keywords** Peri-implantitis, Biomarkers, Peri-implant crevicular fluid, Molecular diagnosis

*Correspondence:
Alejandra Chaparro
Chaparro.ale@gmail.com; achaparro@uandes.cl

Full list of author information is available at the end of the article

## Introduction

Peri-implantitis is a plaque-associated pathologic condition occurring in the surrounding supported tissue of dental implants, characterized by inflammation of the peri-implant mucosa and subsequent progressive loss of supporting bone [1], both of which may contribute to failure and eventually loss of the implants [2]. According to the case definition, peri-implantitis prevalence can reach up to 20% [3] and is aggravated by host-related factors such as smoking, poor oral hygiene, periodontitis history, poorly controlled diabetes, and quiet compromise to peri-implant supportive care [4]. Compared to periodontitis, peri-implantitis displays an acceleratory and non-linear pattern of progression. Moreover, the extension of the immune inflammatory infiltrate within peri-implant tissues has been reported being three-fold higher than in periodontitis [5–7]. Nevertheless, implant-related factors such as prosthetic rehabilitation, implant surface and the emergence profile, may increase the risk of having less reproducible clinical probing measurements challenging the clinical diagnostic [8, 9]. However, despite the differences between both diseases, the same clinical measures such as probing depth (PD), bleeding on probing (BOP), suppuration (SUP), and radiographic bone loss, which are conventionally used for periodontitis diagnosis are also commonly used for the peri-implantitis diagnosis. Thus, we propose the development of molecular diagnosis tools, specifically by identifying specific biomarkers signatures from the peri-implant crevicular fluid (PICF), as a promissory field to develop more accurate diagnostic methods for peri-implantitis risk assessment, especially in the era of precision dentistry [10, 11].

Biomarkers are biological molecules found in host fluids or tissues indicating signs of normal or abnormal processes, conditions, or diseases [12]. Thus, they are helpful for diagnosis, severity assessment, and prognosis determination. PICF, is a serum-derived exudate enriched in host-derived components such as tissue degradation products, immune cells, antibodies, cytokines, which are potentially valuable as biomarkers [13]. Therefore, molecular analysis of PICF have been proposed as a proper technique to analyze inflammatory processes during peri-implantitis and monitor peri-implant conditions, thus becoming a potential precision or personalized diagnostic tool for implantology in clinical practice [10, 11, 14].

Peri-implantitis is mediated by a complex biofilm that colonizes the implant and its surrounding tissues, generating an excessive inflammatory response in the host [1]. Thus, the immune cells that infiltrate the peri-implant tissues have been associated with PI [15]. Consequently, it is suggested that molecules released by activated immune cells might be enriched in peri-implantitis and potentially serve as diagnostic biomarkers of this disease [16, 17].

Th17 cells promote the receptor activator of nuclear factor-kappa B ligand (RANKL) expression on osteoblasts and stimulates osteoclast differentiation, fostering bone resorption [18, 19]. In the same scenario, Interleukin (IL)-23 [20, 21], produced by dendritic and epithelial cells supports the Th17 cell differentiation [21]. In addition, a proliferation-inducing ligand (APRIL) is a cytokine implicated in B cells growth, differentiation, and cell survival [22, 23]. APRIL is produced by myeloid cells, neutrophils, dendritic cells, monocytes [24, 25], and tissue-resident cells [26, 27]. In inflammation, APRIL promotes the maintenance of B cell functions and T cell survival [28–30]. Recent studies showed an increased concentration of APRIL in peri-implant tissues from patients diagnosed with peri-implantitis [31]. Therefore, we hypothesized that these pro-resorptive/proinflammatory molecules may be consistently upregulated during peri-implantitis and could indicate the disease onset. The primary objective of this study was (1) to evaluate PICF levels of APRIL, IL-23, and RANKL from healthy, mucositis and peri-implantitis subjects and, (2) to determine the diagnostic performance accuracy of these biomarkers for the discrimination of peri-implantitis diagnosis.

## Materials and methods

### Study design and participants

A cross-sectional study was conducted in the Health Care Centre of the Universidad de Los Andes, Santiago, Chile, and the Dental School of the Universidad de La Frontera, Temuco, Chile. The present study was approved by the Universidad de la Frontera Scientific Ethics Committee (ID0242018) and was conducted in accordance with the Helsinki declaration of 1973, as amended in 2024. The study was explained to all the participants who signed an informed consent form.

For enrolment, patients had to have at least one dental implant in mouth with a minimum of one year in function, definitive rehabilitation, 16 teeth, no previous implant debridement treatment, or antecedents of peri-implantitis and no prescription of topical/systemic antibiotics or anti-inflammatory therapy during the 3 months prior to the recruitment and diagnosis. Exclusion parameters were chronic inflammatory diseases such as: type 1 or 2 diabetes, autoimmune disease, heavy smokers (more than 10 cigarettes a day), and active infections. A complete periodontal / peri-implant full-mouth exam was performed by one periodontist (A.CH), evaluating periodontal probing depth (PPD), clinical attachment level (CAL), peri-implant probing depth (PIPD), bleeding on probing (BOP), suppuration (SUP), plaque index score (visible plaque accumulation), measured along the gingival o peri-implant mucosal margin and recorded as presence (+) or absence (-) [32]. All relevant sociodemographic/clinical data and sampling of the subjects were

recorded in a database. The analyzed variables analyzed were age, gender, smoking status, history of periodontitis, current periodontal diagnosis, implant location and survival, PPD mean, CAL mean, PIPD mean, BOP, SUP, PIx, GI. Furthermore, we analyzed the levels of APRIL, RANKL, and IL-23 in PICF samples.

**Clinical trial number** Not applicable.

### Diagnostic criteria

The 2017 classification of periodontal and peri-implant diseases and conditions [2], provides clear definitions for healthy implants, peri-implant mucositis, and peri-implantitis as follows:

**Healthy implant** Was characterized by absence of erythema, swelling, BOP, and SUP, and probing depths without showing progression over time. Radiographically, there were no evidence of bone loss beyond the initial remodeling that typically occurs after implant placement. **Peri-implant mucositis** was defined with the presence of clinically, erythema, swelling, BOP and/or suppuration. Radiographic evaluations show no progressive bone changes. **Peri-implantitis** was defined by inflammation in the peri-implant tissues accompanied by progressive loss of supporting bone. Clinically, exhibit inflammatory signs, BOP, and/or SUP, and increased probing depths and/or recession of the mucosal margin in addition to radiographic bone loss compared to previous examinations [2].

### Sample size calculation

Due to the exploratory nature of the present study, we not estimating a sample size, and we arbitrarily established near to 20 patients in each group, with at least one rehabilitated implant in function (healthy, peri-implant mucositis and peri-implantitis implants).

### Peri-implant crevicular fluid (PICF): collection and elution protocol

PICF samples were obtained from four sites per implant site (facial, lingual, mesial, and distal surfaces), were collected by placing paper strips between 3 and 8 mm in depth into de peri-implant sulcus/pocket for 30 s according to the implant clinical diagnosis, in a different day from the complete full-mouth periodontal / peri-implant exam. The four paper strips were pooled and stored in an Eppendorf tube of 1.5 ml at -80ºC until de elution protocol. Paper strips that had been contaminated with saliva or blood were discarded and excluded from the analysis. Cases where participants had more than one implant, we prioritized sampling from implants diagnosed with peri-implantitis or peri-implant mucositis. Only one implant per participant was included in the analysis to avoid intra-individual variability and maintain independent observations across the study population. The supra mucosal plaque was handily and carefully removed with curettes and gently dried with an air syringe. For the elution of PICF samples, the pooled paper strips were placed into 160 µl of PBS with a protease inhibitor cocktail. Then, the samples were vortexed and incubated on ice for 30 min following by a centrifugation at 12,000 x g for 5 min at 4 ˚C. All eluted paper strips were collected into a new tube and placed on ice. The complete elution protocol was repeated, and both eluates were pooled and stored at -80˚C for further analysis.

### Luminex assay

APRIL, IL-23, and RANKL concentrations in PICF samples were quantified using a commercially available custom-designed multiplex Luminex assays kits (R&D Systems, Minneapolis, MN, USA. Catalog #LXSAHM-05, and #LXSAHM-01, respectively) for human APRIL (7,95 – 20,930 pg/mL range detection), IL-23 (11,4–31,830 pg/mL range detection) and RANKL (4,7–11,680 pg/mL range detection), according to manufacturer´s instructions. All samples were analyzed in duplicate. Briefly, the microsphere cocktail (50 µl) was added to each well of a 96 well black plate. PICF elute (50 µl) was added to each well. The plates were carefully covered with an aluminum foil plate sealer an incubated at room temperature for 2 h in a horizontal orbital plate agitator at 800 rpm. Plates were then placed in a specially designed magnet plate holder for 1 min and the liquid was discarded. Each well was washed with 100 µl of buffer for 1 min in the magnet plate and then the liquid was discarded again. Biotin antibody cocktail (50 µl) was added to each well, sealed an incubated at room temperature with agitation for 1 h. Then, each well was washed and diluted in Streptavidin-PE (50 µl). The plate was incubated for 30 min and washed, followed by a re-suspension of the microspheres in 100 µl of buffer and incubation for two minutes. Finally, samples were read using MAGPIX system. The final concentration was calculated using a Milliplex Analyst (version 5.1; Merk KGaA, Darmstadt, Germany).

### Statistical analysis

Absolute frequencies and percentages were used for categorical variables and the median and interquartile range for continuous variables. The Kruskal Wallis test compared continuous variables according to peri-implant status and the exact Fisher test for categorical variables, followed by Dunn´s test for post hoc comparisons. Spearman correlation coefficient was calculated between the clinical parameters (PPD, CAL, PIPD, BOP and Plx) and the biomarkers (RANKL, APRIL, and IL-23). The relationship between the level of standardized biomarkers

and the presence of peri-implantitis was performed through logistic regression models. The crude odds ratio (OR), 95% confidence interval, and *p*-values (significant level $p < 0.05$) were reported. The area under the curve (AUC) was calculated into receiver operating characteristic (ROC) curves. The variables with a *p*-value < 0.05 and an AUC higher than 0.6 were selected through the stepwise forward method. Youden criteria were used to determine cut-off points of the score, and the sensitivity, specificity, predictive values, and likelihood ratios were presented. The analysis was made with STATA (version 16.1; StataCorp, College Station, TX, USA).

## Results

### Demographics data

The present study enrolled fifty-four patients; with 17 healthy implants, 19 peri-implant mucositis and 18 implants diagnosed with peri-implantitis. Demographic, periodontal, and peri-implant data from the participants by implants diagnosis are presented in Table 1. The subjects' ages ranged from 48 to 77 years old, and the studied population showed no significant differences in sex distribution, smoking, periodontal status, implant location, or implant survival. Of note, patients diagnosed with peri-implant disease had higher frequencies of periodontitis history ($p = 0.045$) and significative differences in periodontal status ($p = 0.049$). Peri-implantitis affected implants had a significantly higher plaque index score ($p < 0.001$), implant probing depth mean ($p < 0.001$) and bleeding on probing ($p < 0.001$) compared to peri-implant mucositis and healthy implants.

### Diagnostic accuracy of bone-related biomarkers on peri-implantitis

Thereafter, we analyzed the concentration of IL-23, RANKL, and APRIL in PICF samples, and implants with peri-implantitis had significantly increased of RANKL and APRIL compared to peri-implant mucositis and healthy implants ($p < 0.001$ *and* $p = 0.005$, respectively) (Fig. 1; Tables 2 and 3). Moreover, we explored correlations between the PICF concentrations of RANKL, APRIL, and IL-23 with periodontal and peri-implant clinical inflammatory parameters. Specifically, the present results suggest positive significant correlations

**Table 1** Demographics and clinical characteristics of the studied population by implant status (healthy, peri-implant mucositis v/s. peri-implantitis diagnosis)

| | | Healthy | Mucositis | Peri-implantitis | *p*-value* |
|---|---|---|---|---|---|
| | | *n* = 17 | *n* = 19 | *n* = 18 | |
| Age (years) | | 68.0 (48.0–78.0) | 75.0 (48.0–77.0) | 66.0 (54.0–69.0) | 0.69 |
| Sex | Female | 12 (71%) | 12 (63%) | 11 (61%) | 0.88 |
| | Male | 5 (29%) | 7 (37%) | 7 (39%) | |
| Smoking | No | 11 (65%) | 13 (68%) | 14 (78%) | 0.75 |
| | Yes | 6 (35%) | 6 (32%) | 4 (22%) | |
| Previous history of periodontitis | No | 13 (76%) | 7 (37%) | 8 (44%) | **0.045** |
| | Yes | 4 (24%) | 12 (63%) | 10 (56%) | |
| Periodontal condition | | | | | |
| | Healthy | 13 (76%) | 7 (37%) | 7 (39%) | **0.049** |
| | Gingivitis | 0 (0%) | 0 (0%) | 1 (6%) | |
| | Mild periodontitis | 2 (12%) | 2 (11%) | 0 (0%) | |
| | Moderate periodontitis | 1 (6%) | 5 (26%) | 3 (17%) | |
| | Severe periodontitis | 1 (6%) | 5 (26%) | 7 (39%) | |
| Implant location | Maxillary | 13 (76%) | 10 (53%) | 12 (67%) | 0.35 |
| | Mandibular | 4 (24%) | 9 (47%) | 6 (33%) | |
| Implant survival (months) | | 60.0 (12.0-120.0) | 48.0 (24.0–88.0) | 48.0 (36.0–72.0) | 0.98 |
| Implant suppuration | No | 16 (94%) | 13 (68%) | 15 (83%) | 0.16 |
| | Yes | 1 (6%) | 6 (32%) | 3 (17%) | |
| Periodontal probing depth (full mouth mean, mm) | | 3.4 (3.4–4.1) | 3.9 (2.5–4.2) | 3.7 (2.8–5.3) | 0.92 |
| Clinical attachment loss (full mouth mean, mm) | | 4.3 (3.5–4.3) | 3.6 (2.8–5.5) | 4.6 (4.0-8.2) | 0.098 |
| Bleeding on probing (full mouth %) | | 41.0 (18.0–45.0) | 41.0 (24.0–60.0) | 50.0 (44.0–70.0) | **0.005** |
| Plaque index score (full mouth %) | | 20.0 (13.0–28.0) | 35.0 (28.0–37.0) | 50.0 (44.0–89.0) | **< 0.001** |
| Peri-implant probing depth. (four sites mean, mm) | | 2.6 (2.4–2.8) | 3.0 (2.6–3.2) | 3.9 (3.0-6.5) | **< 0.001** |

*Data are presented as median (IQR) for continuous measures and n (%) for categorical measures.*

**Kruskal Wallis test (continuous) or exact Fisher test (categorical)*

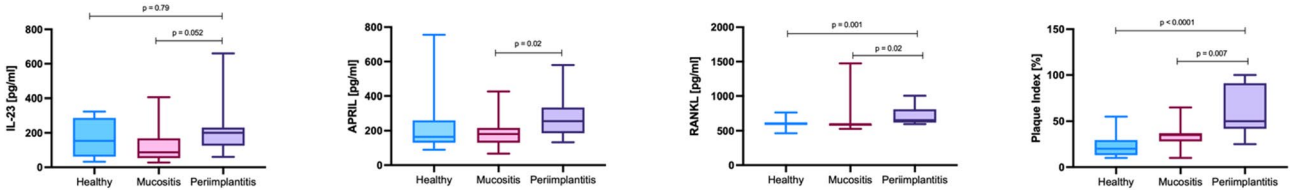


**Fig. 1** Peri-implant crevicular fluid concentrations of IL-23, APRIL, RANKL, and plaque index score description by implant diagnosis. **Kruskal Wallis and Dunn´s test*

**Table 2** Peri-implant crevicular fluid concentrations of IL-23, APRIL, and RANKL (pg/mL) description (median and interquartile range) by implant diagnosis

| Biomarker | Healthy implants | Mucositis | Peri-implantitis | *p*-value* |
|---|---|---|---|---|
| | ***n*** **= 17** | ***n*** **= 19** | ***n*** **= 18** | |
| IL-23 (pg/ml) | 153.5 (61.6–287.0) | 86.8 (52.7-168.1) | 200.1 (127.4-227.7) | 0.060 |
| APRIL (pg/ml) | 164.1 (138.6-251.3) | 181.3 (130.2-216.1)[a] | 268.9 (216.1-400.5)[b] | **0.005** |
| RANKL (pg/ml) | 606.8 (584.1-612.5)[a] | 595.4 (572.7-595.4)[b] | 652.2 (629.5-765.6)[b] | **< 0.001** |

**Kruskal Wallis test. Results are expressed in median and interquartile ranges. Bold*: significantly p-value < 0.05. Different letters per row indicate statistical significance difference with Dunn's Test*

**Table 3** Regression model expressed as Od ratio (OR), *P*-value and confidence interval (95%) of the association between standardized biomarkers in peri-implant crevicular fluid and plaque index score with peri-implantitis diagnosis (continuous variables are standardized)

| Variable | OR | 95% CI | *p*-value |
|---|---|---|---|
| IL-23 (pg/ml) | 1.83 | [0.94–3.57] | 0.076 |
| APRIL (pg/ml) | 3.01 | [1.08–8.38] | **0.035*** |
| RANKL (pg/ml) | 1.55 | [0.83–2.87] | 0.165 |
| Plaque Index score | 11.24 | [2.63–48.16] | **0.001*** |

*Bold*: significantly p-value < 0.05. The results are expressed as od ratio, p-value, and confidence interval (95%) for each variable.*

between PICF-RANKL levels and clinical attachment loss (rho = 0.33, $p < 0.05$), plaque index score (rho = 0.35, $p < 0.05$) and bleeding on probing (rho = 0.33, $p < 0.05$). In addition, PICF-IL-23 levels were correlated with the plaque index score (rho = 0.28, *$p < 0.05$).* Interestingly, PICF-APRIL levels were significant correlated with the peri implant probing depth (rho = 0.28, $p < 0.05$), that could be related to the severity of bone loss and peri-implantitis (Fig. 2). Subsequently, we evaluated the diagnostic performance of each biomarker (Fig. 3). The biomarkers that showed by themselves acceptable discrimination for peri-implantitis diagnosis (AUC-ROC curve > 0.70) were APRIL and RANKL (0.77 and 0.81, respectively). In addition, APRIL levels in PICF demonstrated a robust association with peri-implantitis diagnosis (OR: 3.01; 95% CI [1.08–8.38], $p = 0.035$). Besides, among the clinical parameters, a higher plaque index score was also significantly associated with peri-implantitis diagnosis (OR:11.24; 95% CI [2.63–48.16]; $p = 0.001$). Thus, both variables (PICF-APRIL concentrations and plaque index score values), were included in a bootstrapping stepwise regression model to be tested in a peri-implantitis diagnostic model. The model showed an AUC-ROC curve discrimination of 0.95 for peri-implantitis diagnosis (Fig. 2). The sensitivity corresponds to 94.4%, and the specificity to 83.3% (Table 4). The cut-off points were found by estimating Youden's index for PICF-APRIL, and the plaque index score were − 10.1412 + 0.0109 x APRIL + 0.1527 x plaque index, generating a score with the best cut-off by Youden index (0.778) of -0.8164.

## Discussion

The present study highlights the potential of peri-implant crevicular fluid (PICF)-derived biomarkers, particularly APRIL, for diagnosing peri-implantitis. Our findings show that levels of APRIL and RANKL, were significantly elevated in implants diagnosed with peri-implantitis compared to healthy or peri-implant mucositis implants. Moreover, the present results suggest positive and significant correlations between RANKL levels and clinical attachment loss, plaque index score and bleeding on probing. In addition, IL-23 levels were correlated with the plaque index score. Interestingly, APRIL levels were significant correlated with the peri implant probing depth, that could be related to the severity of bone loss and peri-implantitis. Likewise, the inclusion of APRIL levels in a diagnostic model, alongside clinical inflammatory parameters such as plaque index score, demonstrated a suitable diagnostic accuracy (AUC-ROC curve 95%), supporting its utility as a molecular diagnostic tool for peri-implant diseases.

A growing body of research has highlighted the potential of PICF-derived molecules as biomarkers for peri-implantitis, emphasizing their utility in clinical diagnostics [9–12, 17, 33, 34]. Currently, the diagnosis of peri-implantitis often occurs at an advanced stage, when severe inflammatory lesions and bone loss have already

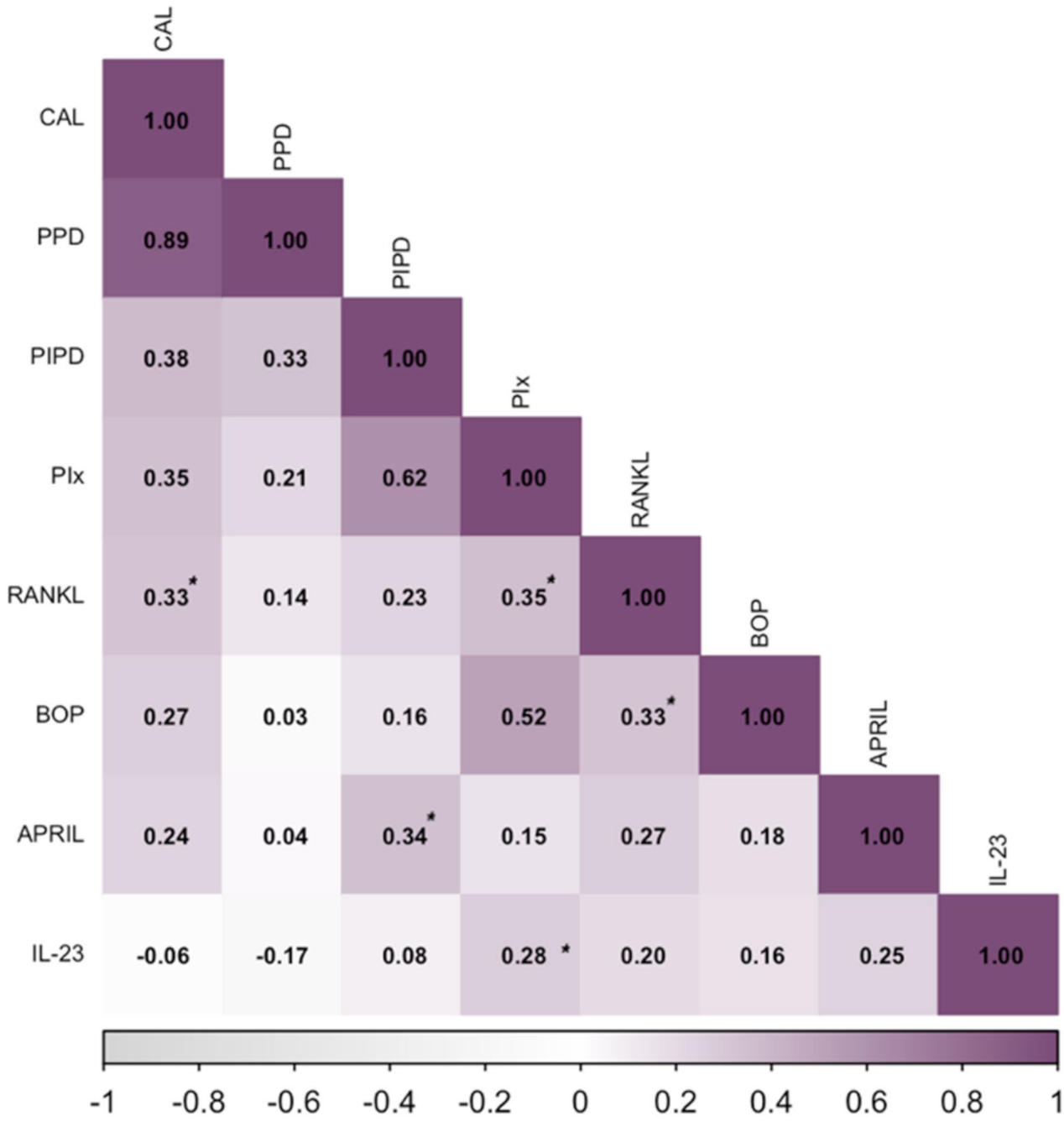


**Fig. 2** Heatplot of correlations between the concentrations of IL-23, APRIL, and RANKL in peri-implant crevicular fluid with periodontal and peri-implant clinical parameters. *Abbreviations: CAL, Clinical attachment loss; PPD, Periodontal probing depth; PIPD, Peri-implant probing depth; PIx, Plaque index score; BOP, Bleeding on probing. Spearman correlation coefficient. *: significantly p-value < 0.05*

developed, making therapeutic outcomes less favorable. Moreover, conventional clinical tools such as bleeding on probing, suppuration, and peri-implant probing depth lack the precision, objectivity, and sensitivity required for early and accurate diagnosis in the asymptomatic stages of the disease. This underscores the critical need for modern implant dentistry to adopt precision molecular implantology and minimally invasive approaches. These approaches, which integrate molecular diagnostics and predictive algorithms combining risk factors, clinical variables, and molecular biomarkers, hold promise for improving diagnostic accuracy in the era of precision and personalized dentistry [35]. In fact, the role of host-derived inflammatory molecules in oral fluids as

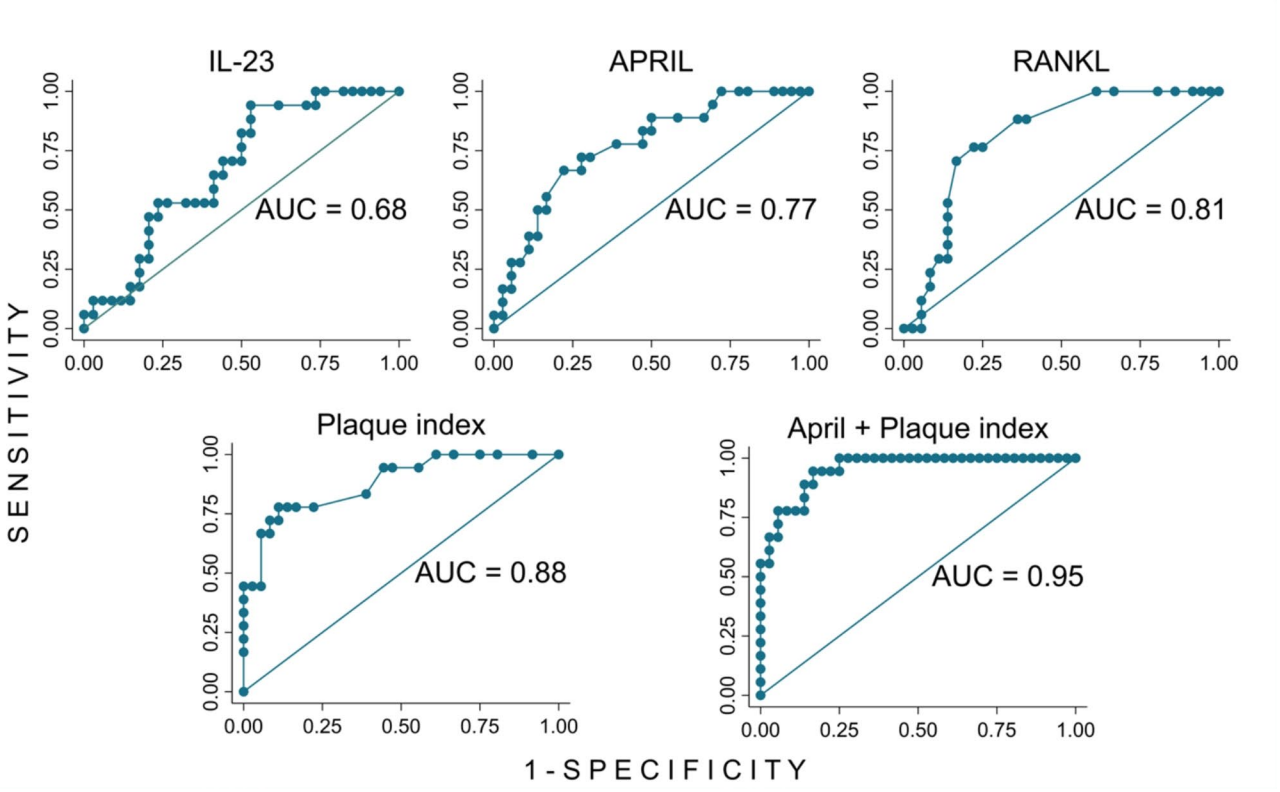


**Fig. 3** Area under the AUC-ROC curve for the studied biomarkers in peri-implant crevicular fluid and plaque index score, and the proposed model which combined peri implant fluid levels of APRIL with the plaque index score for the peri-implantitis diagnosis discrimination

**Table 4** Cut-off performance of the score through the diagnostic regression model that combines the PICF-APRIL concentrations with the plaque index score

| | | 95% CI | |
|---|---|---|---|
| Sensitivity | 94.4 | 72.7 | 99.9 |
| Specificity | 83.3 | 67.2 | 93.6 |
| Likelihood ratio + | 5.67 | 2.71 | 11.9 |
| Likelihood ratio - | 0.067 | 0.01 | 0.45 |
| Positive predictive value | 73.9 | 51.6 | 89.8 |
| Negative predictive value | 96.8 | 83.3 | 99.9 |

complementary biomarkers for peri-implantitis is an area of active research. Molecular and bioinformatics-based strategies are poised to transform peri-implant health diagnosis and monitoring, enabling earlier detection during the pre-symptomatic phase or at the early onset of peri-implantitis, when clinical signs are not yet evident [35].

Peri-implant bone loss results from a combination of factors, with inflammation playing a central role [1, 2, 5, 34]. The release of pro-inflammatory cytokines in the peri-implant tissues can overwhelm the local defensive and regenerative capacities, leading to tissue breakdown [33–36]. A key contributor to this process is the imbalance between RANKL and osteoprotegerin (OPG), which drives increased catabolic activity and bone resorption through osteoclast overstimulation [37–39]. Consistent with these findings, elevated levels of PICF-RANKL have been observed in patients with peri-implantitis in other studies, supporting the results of this research [40, 41]. For instance, while RANKL has been detected during peri-implant mucositis, it has shown limited ability to distinguish between mucositis and peri-implantitis on its own. However, when combined with clinical parameters such as bleeding on probing and probing depth in diagnostic models, RANKL significantly enhanced the ability to discriminate between healthy peri-implant tissues, peri-implant mucositis, and peri-implantitis [10].

Peri-implantitis involves plasma cells as a predominant inflammatory cell type. In this sense, APRIL, associated with B-cell recruitment and homeostasis, is expected to be elevated during inflammation [5, 15]. In the present study, APRIL was the most upregulated molecule with an improved diagnostic performance discriminating between peri-implantitis and healthy/peri-implant mucositis implants. This result agrees with our previous findings, showing a higher expression of APRIL within the peri-implantitis affected soft tissues by immunohistochemistry [31]. Additionally, both APRIL mRNA and protein levels have been reported to be upregulated in periodontitis, which correlates with increased amount of B and plasma cells. In the same context, RANKL

overexpression was correlated with an APRIL increase [28]. B cells are also major producers of RANKL in bone resorptive lesions during periodontitis [38]. It is likely that APRIL contributes to bone loss during peri-implantitis development. A recent preclinical study showed that RANKL-expressing B cells increased only around implants compared to T cells, which were also augmented in the contralateral periodontal tissue with no implant placement [42].

IL-23, a crucial cytokine in the proliferation of the Th17 lineage has been implicated in peri-implant inflammation, and it is known that the IL-23/IL-17 axis is highly present in periodontal and peri-implant tissues contributing to a destructive inflammatory environment [43, 44]. IL-23 are usually over expressed in situations of bacterial insults and are responsible to detect infections and coordinating the pathogens elimination that damage periodontal and peri-implant tissues [45, 46]. Although, the increase in this cytokine was not significant in the present study, we did observe a tendency towards an increase in peri-implantitis affected implants. These findings are aligned with the results of recent studies [46, 47]. Probably, the increase in IL-23 may lead to a faster rate of PIPD progression and impaired osseointegration with the subsequent implant failure. However, we need more research on the role of IL-23 in peri-implant disease.

Summarizing, the most significant contribution of this study is the potential use of PICF-APRIL levels as a biomarker for clinical usefulness in peri-implant disease diagnosis, laying the groundwork for developing non-invasive tools for its precision and personalized implant molecular diagnosis. However, the findings must be interpreted with caution, considering several limitations. First, the study's cross-sectional design restricts our ability to establish causal relationships between the biomarker's concentration and peri-implant disease progression. Longitudinal studies are necessary to confirm whether elevated levels of APRIL and other biomarkers precede peri-implantitis or simply reflect existing disease. Second, the sample size was relatively small, which may limit the generalizability of the findings, and future studies with larger and more diverse populations are needed to validate these results and refine the diagnostic thresholds. Third, it would also be interesting to determine their correlation with plasma levels and their response to peri-implant therapy. Fourth, the cost of determining biomarkers is still relatively expensive and the implementation of this technology must be scaled to the dental clinic scenario. Finally, while the observed associations between elevated APRIL levels and peri-implantitis provide promising evidence for its diagnostic potential, the lack of microbiological evaluation does not allow to assess the interplay between the local microbiota and the host immune-inflammatory response in peri-implantitis affected implants. Future studies incorporating both, molecular biomarker profiling and microbial characterization are essential to gain a comprehensive understanding of the pathogen–host interactions driving peri-implant disease progression.

The present study lays the groundwork for the development of non-invasive, precision diagnostic tools for peri-implantitis using molecular biomarkers. Future prospective longitudinal studies which explore the temporal dynamics of the biomarker's expression, both, baseline and after peri-implant therapy, will allow to establish robust diagnostic algorithms that integrate clinical, radiographic, and molecular data to screening the health of peri-implant tissues. Such advancements will enhance the early detection and personalized management of peri-implant diseases in clinical practice. In this sense, we propose that PICF-derived immune inflammatory components as promising tools that could significantly impact molecular diagnosis and the future development of precision implant dentistry, enabling personalized clinical risk assessment.

## Conclusions

Implants diagnosed with peri-implantitis display higher levels of APRIL and RANKL in PICF samples compared to healthy/peri-implant mucositis implants. A multivariate model which includes the PICF-APRIL levels combined with plaque index score leads to an enhancement discrimination of peri-implantitis. Thus, findings from this study highlight the potential precision and personalized molecular approach to primary/secondary prevention of peri-implant disease.

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#### Author contributions

ACH, JN, AP, AM and VB conceived the idea, design the clinical study and were involved in writing the manuscript. MJB, CR processed samples and performed lumina assays, VR y DA performed statistical analysis, All the authors contributed to the article by reviewing and editing.

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#### Data availability

All the relevant information from the study is described in the manuscript.

## Declarations

#### Ethics approval and consent to participate

The Ethical Committee boards of the Universidad de la Frontera approved the study (#ID0242018). Detailed explanation of the project and a written informed consent was given and obtained from all study participants. The present research was conducted in compliance with the Helsinki declaration of 1973, amended in 2024.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Author details**
[1]Department of Oral Pathology and Conservative Dentistry, Faculty of Dentistry, Universidad de Los Andes, Santiago, Chile
[2]Department of Sciences and Research, Epidemiology and Statistics, Faculty of Dentistry, Universidad de Los Andes, Santiago, Chile
[3]Centro de Investigación e Innovación Biomédica (CIIB), Periodontal Research laboratory (LIP-LAB), Universidad de los Andes, Santiago, Chile
[4]Department of Periodontology, School of Dentistry, Universitat Internacional de Catalunya, Barcelona, Spain
[5]Clinical Investigation and Dental Innovation Center (CIDIC), Dental School, Center for Translational Medicine, Universidad de La Frontera, Temuco, Chile

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## Publisher's note