Biomarkers from Peri-Implant Crevicular Fluid

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Some inflammatory biomarkers harvested from peri-implant crevicular fluid (PICF) (collagenase-2, collagenase-3, ALP, EA, gelatinase b, NTx, procalcitonin, IL-1β, and several miRNAs) seem to be correlated with peri-implant bone loss (BL) and may assist in the early diagnosis of pathological BL, that characterizes peri-implantitis. MiRNA expression demonstrated a predictive potential of peri-implant BL that could be useful for host-targeted preventive and therapeutic purposes. PICF sampling may represent a promising, noninvasive, and repeatable form of liquid biopsy in implant dentistry.

dental implants bone loss biomarkers peri-implant crevicular fluid

1. Introduction

Fixed implant-supported restorations are extensively employed to rehabilitate partially or completely edentulous patients with predictable outcomes. However, despite their high percentages of success, dental implants are not free from possible complications with consequent failure, the causes of which are still the object of debate in the dental scientific community.

In particular, peri-implant infections are multifactorial pathological conditions characterized by peri-implant mucosal inflammation with or without progressive loss of supporting bone (i.e., peri-implantitis or peri-implant mucositis, respectively) [1]. Peri-implantitis may be asymptomatic or may appear clinically as mucosal erythema, edema, increased probing depth (PD), bleeding on probing (BOP) with eventual suppuration, and nonlinear progressive bone loss (BL) [2].

The diagnosis of peri-implantitis, especially in its early phases, is crucial in order to prevent the need to treat an active pathology, as an effective and predictable treatment protocol has not been universally validated [3]. In addition, diagnosis of peri-implantitis is not easy, and different criteria have been proposed by different authors [4][5]. According to the last ITI Consensus Report, the presence of BOP is not always predictable for the presence of peri-implantitis, and BOP alone is insufficient for making a diagnosis [6].

Furthermore, probing an implant can be useful to monitor PD, but it may be insufficient to determine the extent and pattern of BL over time without radiographs [3].

Indeed, the most frequently used definition of peri-implantitis considers it an “inflammatory reaction associated with loss of supporting bone tissue around an implant” [7]. Accordingly, the new definition of peri-implantitis proposed by

Renvert et al. was based on the concomitant presence of peri-implant signs of inflammation and radiographic BL following initial healing [8].

However, radiographical peri-implant bone level assessment is not always predictable and presents several limitations, including that only mesial and distal BL can be evaluated in periapical and panoramic radiographs. Dedicated software can be employed to measure bone level change, as well as implant length can be used to correct the radiographic distortion. However, it is possible that not all lesions will be identified, leading to a lack of sensitivity [9].

Moreover, even if all clinical parameters and changes in bone levels were combined, they may not be sufficient to predict the patient's risk of developing peri-implantitis and its prognosis at the beginning of the inflammation process [10]. For this reason, early diagnosis of pathologic BL and identification of early biomarkers for a peri-implant disease are essential. Diagnosis could be implemented by detecting immunological host-derived molecules, such as chemokines, cytokines, bone markers, and enzymes involved in peri-implant tissues turnover [11]. Biomarkers such as pro-inflammatory cytokines (i.e., tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-17 (IL-17)) are classically associated with the initiation of the inflammatory cascade. They have also been proven to be stimulated by periodontal pathogens' virulence. Other substances, including neutrophil elastase collagenase, alkaline phosphatase, and aspartate aminotransferase, have been weakly associated with peri-implantitis [12]. Markers of bone tissues (i.e., osteoprotegerin (OPG) and soluble receptor activator of nuclear factor kappaB Ligand (sRANKL)), osteoclastogenic-related cytokines and chemokines (i.e., granulocyte colony-stimulating factor (G-CSF), matrix metalloproteinase-8 (MM-8), monocyte chemoattractant protein (MCP-1) are other important molecules which could be considered to understand better the immune-inflammatory profile of peri-implant disease [13].

More recently, thanks to the development of genomics and epigenomics, other classes of biomarkers, which may help identify individual susceptibility, have been considered for studying multifactorial and complex diseases. MicroRNAs (miRNAs), for example, are small endogenous sequences of noncoding RNAs (ncRNAs) responsible for specific regulation of gene expression in a post-transcriptional manner [14]. They are involved in biological processes, such as immune-inflammatory response, bone metabolism, cell replication, and apoptosis [15]. They are already extensively employed for the early diagnosis, prognosis, and personalized therapies of oncologic and genetic diseases, but they have still been scarcely explored in dental implantology [16].

Interestingly, specific expression profiles of miRNAs extracted from peri-implant tissues have been reported to be predictive of specific clinical outcomes of dental implants and may be used as biomarkers in implant dentistry with diagnostic and prognostic purposes [14][17][18]. Although a mini-invasive sample of peri-implant tissue might be sufficient, this procedure shows a certain degree of invasiveness.

It is thought that the detection of biomarkers in several biologic fluids may be a predictable surrogate of traditional tissue biopsies for diagnosis and prognosis of inflammatory processes, and it has been demonstrated that peri-

implant disease could be effectively assessed by the analysis of peri-implant crevicular fluid (PICF) from the peri-implant pocket [19][20].

In the last decades, several studies have shown the presence of host-derived biochemical mediators in PICF, and levels of these inflammatory molecules have been proposed as a measure of active peri-implantitis. Biomarkers assessment in PICF may also be useful to identify specific markers responsible for the onset and development of peri-implantitis since its earliest stages when it is still clinically latent.

As a further advantage, PICF is also a site-specific and easily collectible biofluid that could be valuable for the examination of immunological biomarkers by a noninvasive method, that might be repeated over time, besides the fact that peri-implantitis is usually accompanied by an increased volume of PICF [21].

2. Bibliographic Search and Study Selection

The initial search strategy provided a total of 158 articles: 101, 17, and 40 articles were found on PubMed/MEDLINE, Cochrane Library, and Google Scholar databases, respectively. After eliminating all duplicates, 127 possibly relevant studies were detected. In fact, a total of 31 duplicate articles were removed before the screening. After screening the articles' titles and abstracts, 38 possibly relevant studies were detected for full-text examination. The final selection after full-text analysis included nine papers [12][14][22][23][24][25][26][27][28]. A flowchart was drawn up describing the results of the study search and selection.

3. Included Studies

The nine included studies have been conducted from 2000 to 2022. One of the included studies was conducted in Turkey [24], two in Finland [22][23], one in Sweden [27], one in Italy [25], one in Saudi Arabia [28], one in Japan [26], one in Switzerland [14], and one in Hungary [12]. All the studies were written in English.

Detailed information for the nine included studies is reported in **Table 1**.

Table 1. Main characteristics of included studies.

Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
Ma et al., 2000 [22]	Cross-sectional study	13	49	Collagenase-2 Collagenase-3	Group 1: BL < 1 mm; Group 2: BL from 1 to 3 mm; Group 3: BL > 3 mm	Time-resolved immunofluorometric assay (collagenase-2) Quantitative immunoblot (collagenase-3)	Collagenase-2 (p = 0.049) and collagenase-3 (p = 0.041) levels were significantly higher in Group 3 than in Groups 1

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Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
							and 2. Collagenase-3 and collagenase-2 produced by adjacent bone osteoclast cells reflect irreversible peri-implant vertical BL around loosening dental implants. Measurements of collagenase-3 and collagenase-2 could be used as markers to indicate the degree of peri-implant vertical BL.
Plagnat et al., 2002 [12]	Cross-sectional study	15	19 (healthy: 11; peri-implantitis: 8)	ALP EA α2M	Healthy implants: no radiographic evidence of BL. Implants with peri-implantitis: crestal BL greater than 20% in at least one site (mesial or distal) along the implant	P-nitrophenyl-phosphate as substrate (ALP) low molecular weight Fluorogenic substrate (EA) ELISA (α2M)	ALP and EA were correlated with the percentage of BL. ALP and EA could be promising markers of BL around dental implants.
Ma et al., 2003 [23]	Cross-sectional study	13	46	Gelatinase b	Group 1: BL < 1 mm; Group 2: BL from 1 to 3 mm; Group 3: BL > 3 mm.	Modified urokinase assay	The differences between activated (p = 0.044) and total gelatinase B (p = 0.026) levels were

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Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
							significant in the three BL groups. Furthermore, gelatinase B levels were increased in Group 3 compared to Groups 2 and 3. Activation of gelatinase B together with elevated mGI eventually reflects active phases of peri-implantitis and may prove to be diagnostically useful.
Yamalik et al., 2012 [24]	Cross-sectional study	40	54 (Group P-1: 19 healthy implants; Group P-2: 27 implants with mucositis; Group P-3: 8 implants with peri-implantitis)	Cathepsin-K	The actual distance between two consecutive threads of the dental implant was used as a reference point	Cathepsin-K activity assay kit	Mean BL values for subgroups P-1, P-2, and P-3 were 1.242, 1.514, and 1.844 mm, respectively ($p = 0.087$). Mean total cathepsin-K activity levels of subgroups P-1, P-2, and P-3 were 3.637, 6.114, and 16.290 units, respectively. However, there is no positive correlation between the enzymatic profile of PICF and the BL measurements.

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Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
							Mean BL around dental implants did not significantly correlate with total cathepsin-K activity.
Yaghobee et al., 2013 [25]	Cross-sectional study	32	41	IL-1β	BL measured by intraoral periapical radiographs	Enzyme-linked immunosorbent assay (ELISA)	It seems that there Is a positive correlation between IL-1β level and BL ($p < 0.0001$) Mean BL: 1.66 mm.
Sakamoto et al., 2018 [26]	Cross-sectional study	35	74 (healthy: 34; peri-implantitis: 40)	Calprotectin and cross-linked N-telopeptide of Type I Collagen (NTx)	BL of more than 2.5 or 3 mm evaluated around dental implants by intra-oral radiographs	Enzyme-linked immunosorbent assay (ELISA)	The mean BL rate of peri-implant disease sites was 42.7%, and that of healthy sites was 19.7%. The BL rate in healthy sites ranged between 6.9 and 41.8%, and that in diseased sites was between 7.7 and 80.0%. A positive correlation was observed between NTx amounts and the BL rate ($\rho = 0.570$, $p < 0.001$). Calprotectin and NTx in PICF are markers of inflammation and BL in peri-

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Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
							implant tissues and may be useful diagnostic markers for peri-implant diseases.
Lira-Junior et al., 2019 [27]	Cross-sectional study	43	42 (mucositis: 20; peri-implantitis: 22)	CSF-1; IL-34; IL-1β	BL measured by intraoral periapical radiographs; mucositis: BL around the implant not reaching the first thread; peri-implantitis: BL involving at least two implant threads	Commercial enzyme-linked immunosorbent assays	There is no statistically significant correlation between CSF-1, IL-34, IL-1β, and BL.
Algohar et al., 2020 [28]	Cross-sectional study	60	94 (healthy: 32; peri-implant mucositis; 27: peri-implantitis: 35	Procalcitonin	BL: radiographic level of bone ≥3 mm apical of the most coronal portion of the intraosseous part of the implant after initial bone remodeling. BL is defined as the linear distance measured from the implant-abutment junction to the most	Enzyme-linked immunosorbent assay (ELISA)	Mean BL in healthy, mucositis, and peri-implantitis groups were, respectively: 0.7 mm (0.5), 1.1 mm (0.6), and 2.5 mm (0.9). In the peri-implantitis group, a significant positive correlation was observed between crestal BL (p = 0.0013) and PICF procalcitonin levels.

BL measurements were calculated from intraoral radiographs in all the included investigations.

Eight of the included studies were cross-sectional studies [12][22][23][24][25][26][27][28]. Only the study by Menini et al. was a prospective cohort study [14].

The following biomarkers were examined: calprotectin, cross-linked N-telopeptides of type I collagen (NTx), cathepsin-K, alkaline phosphatase activity (ALP), elastase activity (EA), inhibitor α2-macroglobulin (α2M),

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Authors	Study Design	Number of Patients	Number of Implants	Assessed PICF Biomarkers	Definition of BL *	Type of Assay	Main Outcomes
					coronal point of the alveolar crest. [14]		
Menini et al., 2021 [20]	Prospective cohort study	7	14 [28] [22][23]	MicroRNAs * [24] [12] [25]	BL was considered normal if it was ≤1 mm and increased if it was >1 mm	Microarray technology [25]	MiRNAs may be used as biomarkers of peri-implant bone resorption. The following miRNAs were altered in the case of BL both in PICF and in soft peri-implant tissues: miR34 a; miR100; miR106 a; miR126; miR143; miR146 a; miR181; miR200; miR221; miR223; miR375; miR378; miR429; miR1248. [29]

In five articles, PICF collection was obtained using paper points left in the sulcus for 30 s [14][24][25][26][27]. In two studies, a filter strip was placed into the sulcus for 4 min [22][23]. In one study, PICF was collected with paper strips left in the peri-implant sulcus for 15 s [12]. Finally, in one study, paper cones were employed, but the insertion time was not specified [28].

In most of the studies, PICF samples were collected at mesial and distal sites. In the study by Lira et al., it was specified that the sites were inflamed with gingival indexes of 1 or 2 [27]. In the studies by Ma et al., the samples were collected from the site with maximum vertical BL and assessed by X-ray [22][23].

Biomarkers collected from PICF were examined through different types of assays: enzyme-linked immunosorbent assay (ELISA) [12][25][26][28], cathepsin-K activity assay kit [24], P-nitrophenyl-phosphate as substrate [12], low molecular weight fluorogenic substrate [12], modified urokinase assay [23], time-resolved immunofluorometric assay, quantitative immunoblot [22], and commercial enzyme-linked immunosorbent assays [27]. MiRNAs were evaluated with microarray technology [14].

Because of the methodological heterogeneity of the included studies, and in particular, because different biomarkers were investigated in the different studies, a meta-analysis was not appropriate and was not conducted.

4. Results of Individual Studies

Most of the studies found a positive correlation between the biomarkers collected from PICF and BL. In the study by Sakamoto et al. [26], the BL rate in healthy sites ranged between 6.9 and 41.8%, while that in diseased sites was between 7.7 and 80.0%; a positive correlation was observed between NTx amounts and BL rate ($\rho = 0.570$, $p < 0.001$). In the study by Algohar et al. [28], implants were divided into healthy and diseased (with mucositis or peri-implantitis), and it was found that mean BL in healthy, mucositis, and peri-implantitis groups were, respectively: 0.7 mm (0.5), 1.1 mm (0.6), and 2.5 mm (0.9). In the peri-implantitis group, a significant positive correlation was observed between crestal BL ($p = 0.0013$) and PICF procalcitonin levels.

In the study by Menini et al. [14], there was a positive correlation between miRNA expression profile and BL. Moreover, 14 miRNAs that were altered in PICF in the case of greater BL were also altered in the soft peri-implant tissue of the same implant sites.

In the study by Ma et al. [22], it was found that collagenase-2 ($p = 0.049$) and collagenase-3 ($p = 0.041$) levels were significantly higher in Group 3 than in Group 1 and 2 (Group 1: BL < 1 mm; Group 2 BL from 1 to 3 mm; Group 3 BL > 3 mm). The authors concluded that collagenase-3 and collagenase-2 produced by adjacent bone osteoclast cells reflect irreversible peri-implant vertical BL around loosening dental implants. In the second study by Ma et al. [23], the differences between activated ($p = 0.044$) and total gelatinase B ($p = 0.026$) levels were significant in the three BL groups. Furthermore, gelatinase B levels were increased in Group 3 compared to Groups 2 and 3. Activation of gelatinase B together with elevated mGI (modified Gingival Index) eventually reflects active phases of peri-implantitis and may prove to be diagnostically useful.

In the study of Plagnat et al. [12], implants were divided into two groups: healthy implants that lost a mean maximum of 0.6 mm of crestal bone, whereas in the diseased group (that is, implants with a BL greater than 20% in at least one site-mesial or distal-along the implant), a correlation was found between EA and the percentage of BL.

Additionally, in the study by Algohar et al. [28], in the peri-implantitis group, a significant positive correlation was observed between crestal BL ($p = 0.0013$) and PICF procalcitonin levels. In fact, a significantly higher level of PICF procalcitonin was found in the implants with BL compared to the groups of healthy implants ($p = 0.039$) and groups of implants with mucositis ($p = 0.042$). Even the study by Yaghobee et al. found a positive correlation between IL-1β levels and BL ($p < 0.0001$) [25].

Only two studies did not find any statistically significant correlation between BL and PICF biomarkers. In Lira J et al., CSF-1, IL-34, and IL-1β were not related to BL [28]. Moreover, in the article by Yamalik et al., the mean BL values for subgroups P-1, P-2, and P-3 were 1.242 mm, 1.514 mm, and 1.844 mm, respectively ($p = 0.087$), and mean total cathepsin-K activity levels were 3.637, 6.114, and 16.290 units, respectively, with no positive correlation between the enzymatic profile of PICF and BL [24].

5. Excluded Studies

Out of 38 papers for which the full text was analyzed, 29 were excluded from the systematic review [13][29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54][55][56]. After full-text reading, the studies were excluded for two main reasons: (1) studies that did not measure BL; (2) studies that did not correlate PICF biomarkers with BL.

6. Quality Assessment

The risk of bias in the included studies was assessed using the Joanna Briggs Institute (JBI) tools. The purpose of this appraisal is to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias. The questions for each checklist and the relative risk of bias are reported in **Table 2** and in **Table 3**.

Table 2. Risk of bias for clinical studies included according to the critical appraisal tools of JBI Scale for analytical cross-sectional studies.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ma et al., 2000 [22]	No	No	Unclear	Unclear	No	No	Unclear	Unclear
Plagnat et al., 2002 [12]	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes
Ma et al., 2003 [23]	Yes	Unclear	No	Unclear	Unclear	Unclear	Yes	Yes
Yamalik et al., 2012 [24]	yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes
Yaghobee et al., 2013 [25]	Yes	Yes	Unclear	Unclear	Yes	Unclear	Yes	Yes
Sakamoto et al., 2018 [26]	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Lira-Junior et al., 2020 [27]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Algohar et al., 2020 [28]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 3. Risk of bias for clinical studies included according to the critical appraisal tools of the JBI Scale for Cohort Studies.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Menini et al., 2021 [20]	Unclear	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes