FJDM | FRENCH JOURNAL OF DENTAL MEDICINE

How useful is saliva in detecting and monitoring periodontal disease? An update

Sarah Gomulinski[1] **and Catherine Bisson**[2] and **Marie Dubar**[1]

1 Department of Periodontology, School of Dentistry, University of Lille, France
2 Department of Periodontology, School of Dentistry, Nancy University Hospital and Lorraine University, France

Corresponding author: Dr. Marie Dubar, Department of Periodontology, School of Dentistry, University of Lille, France. dubar.marie.jp@gmail.com

Abstract:
Introduction: Saliva, as a mirror of oral health, contains organic and inorganic compounds that can be quantified and may become biomarkers. Biomarkers could have a diagnostic, predictive or prognostic value by identifying patients with an increased susceptibility to develop periodontal diseases, the sites with an active disease and the ones which are about to be and/or by monitoring the effectiveness of periodontal treatment.

Objective: The aim of this scoping review is to synthesise current knowledge of saliva's properties in relation to periodontal diagnosis and management.

Methods: 32 articles published in the past ten years were identified from four databases (Medline, Scopus, Web of Science and Cochrane Library) using the PRISMA-ScR methodology.

Results/Discussion: The studied parameters were either periodontopathogens, or molecules of the inflammatory response such as pro-inflammatory cytokines or tissue degradation such as metalloproteinases or a combination of both. Diagnostic, prognostic or predictive value of salivary components have been studied over the past decades, and potential biomarkers of periodontitis have been identified in saliva such as the combination of MMP-8 and IL-6 in early periodontitis diagnosis. To date, the overall reliability of salivary markers remains insufficient to recommend their use in routine practice for the management of periodontal diseases.

Key words: *Saliva, biomarkers, periodontitis, bacteria, inflammation*

Manuscript submitted: 1 March 2021. **Manuscript accepted**: 29 June 2021.
Doi: https://doi.org/10.36161/FJDM.0010

INTRODUCTION

The early diagnosis of periodontal disease, the identification of periodontal risk patients or the prediction of the response to periodontal treatment are challenging for researchers and practitioners. The conventional approach to the positive diagnosis or recurrence of periodontal diseases, and in particular periodontitis, is based on monitoring the absence of degradation of the periodontal condition (absence of progression of attachment loss and/or periodontal pocket depth) using clinical and radiographic measurements. However, these measurements are only a record of tissue loss and do not allow real-time analysis of changes that occur or anticipation of these changes.[1] The pathological processes, leading to the destruction of periodontal tissue, are already activated before they are clinically observable. Thus, early detection of periodontal diseases, and in particular periodontitis, could help to prevent its consequences and complications. Various periodontal risk assessment methods are available for determination of patients' individual risk and the best known is the PRA (Periodontal Risk Assessment). However, the predictive value of these models are limited in patients without periodontitis and without risk factors of periodontitis.[2,3] Moreover, potential inconsistencies have been recently reported between different risk assessment methods.[4] The development and validation of non-invasive diagnostic tools such as those using saliva for the detection of gingival inflammation and early stages of periodontitis (stage I) have become a part of the recommendations for future research advanced in the new classification of periodontal diseases in 2017.[5] Moreover, the detection of non-responsive patients and/or vulnerable sites, would increase the success rate of periodontal therapies.

Crevicular fluid or saliva have been extensively studied as an additional diagnostic or monitoring tool for periodontal disease, due to the accessibility and non-invasiveness of their samplings.[6] Saliva is a unique and abundant oral fluid, consisting of a mixture of 90% of the major salivary glands (parotid, submaxillary and sublingual glands) and 10% of the minor glands (labial, buccal, lingual, palatal glands). This clear and slightly acidic heterogeneous liquid (pH 6.0-7.0) consists of 99% water, 0.3% protein and 0.2% inorganic substances.[7] It also includes

constituents of non-salivary origin derived from crevicular fluid, sputum bronchial secretions, serum, as well as microorganisms and their metabolic products (bacterial, viral and fungal), desquamated epithelial cells and food debris.[8] Individual salivation can vary from 0.3 to 0.7 ml of saliva per minute, for a total of 1-1.5 litres per day. Saliva plays an important role maintaining the tooth integrity and homeostasis of the oral cavity. It contributes to the lubrication of the mucous membranes, the buffering capacity of the dental structures and is involved in the digestive process.[9] It can be considered as a mirror of oral and systemic health.[10,11] Thanks to advances in metatranscriptomics, metagenomics, biochemistry and immunology, several studies have identified and measured a panel of potential biomarkers in saliva, including cells, cellular activities and molecular or microbial constituents.[12,13,14]

There are two methods of saliva collection: (i) unstimulated saliva which is passively collected in a tube from the patient's oral cavity or (ii) stimulated saliva whose production is induced by chewing. The technique of collecting non-stimulated saliva is required for biomarker research in order not to induce a modification of its constituents proportional to the duration of stimulation.[15] Biomarkers are defined as cellular, biochemical, molecular or genetic alterations by which a physiological or pathological process can be recognised or monitored.[16] They can be reliably measured and objectively evaluated as indicators of health, pathogenic processes, environmental exposure and pharmacological responses to therapeutic intervention. The identification of an ideal biomarker of periodontal disease that would be able to (i) detect the risk to develop periodontitis, (ii) reflect its severity, (iii) monitor the response to periodontal treatment, and (iv) predict its prognosis, has been the subject of active research for more than two decades.[17,18,19] In saliva, the biomarkers are molecules whose concentrations are modulated by disease activity. The identified candidate molecules are related to bacterial metabolism, the host's immuno-inflammatory response or the mechanisms of periodontal destruction.[20] The aims of this review are to summarise recent advances on this topic through reported in the literature and to discuss the clinical significance and application prospects of saliva, as a source of biomarkers for the early diagnosis and the prognosis of periodontal diseases.

Methods

This scoping review was conducted in accordance with the preferred reporting elements for systematic reviews and meta-analyses extended to scoping review (PRISMA-ScR) (*Figure 1*).[21] The main question of this review was: how can saliva contribute to the diagnosis and monitoring of periodontal diseases and conditions? In order to answer it, three sub-questions were also posed: can salivary compounds (i) help in the early diagnosis of periodontitis? (ii) predict the progression to periodontitis? or (iii) predict the response to periodontal treatment?

An electronic search was conducted using four databases: Medline (Pubmed), Scopus, Web of Science and Cochrane Library. The key words used were: ("biomarkers" OR "markers") AND ("salivary" OR "saliva") AND ("diagnosis" OR "prognosis") AND ("periodontitis" OR "periodontal disease"). An additional manual search was carried out based on the references of the articles and scientific journals cited in the bibliography of studies. Two authors (MD and SG) independently sought in the selected electronic databases, according to key words previously described, and extracted relevant studies after reviewing the titles and abstracts. A comparison of the selected studies, by each author, was first performed. Then the same two authors performed a full reading of all identified articles and studies that qualified for the following inclusion criteria; studies: (i) published between 2009 and 2020, (ii) involving salivary extracted molecules, (iii) for which the authors identify them as potential diagnostic, prognostic biomarkers of periodontal disease or predictive of periodontal treatment outcomes, (iv) including an adult population with no specific general disease nor systemic condition.

The following non-inclusion criteria were applied: (i) literature reviews or meta-analyses, (ii) studies dealing with salivary

Figure 1

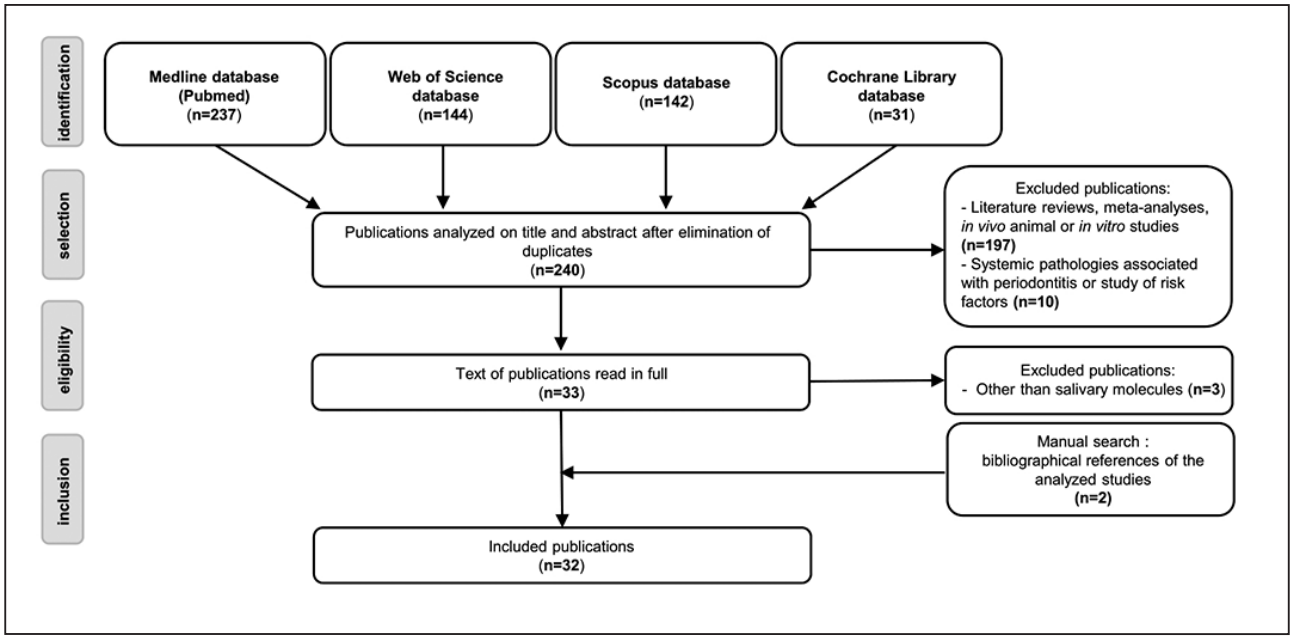


Table 1: Qualitative analysis of included studies

Authors	Scale for qualitative analysis	Score	Identified risk of bias
Hyvärinen *et al.*, 2009 (26)	NOS for case-control studies	56%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear - Representativeness of the study population - Presence of smokers in both groups (57,1% in periodontitis group and 23,5% in control group) without adjustment of results according to the smoking status - Lack of information concerning the presence of systemic diseases or medications in both groups. - No blind periodontal examination and sample processing
Saygun *et al.*, 2011 (27)	NOS for case-control studies	78%	- Lack of information on the smoking status of patients in the different groups - No blind periodontal examination and sample processing
Yamanaka *et al.*, 2012 (56)	NIH quality assessment tool for before-after (Pre-post) study with no control group	50%	- Definition of cases based on unspecified periodontal classification - Lack of information on the smoking status of study's participants - No blind periodontal examination and sample processing
Salminen *et al.*, 2015 (29)	NOS for case-control studies	44%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear and subjects in control group are disparate with patients with mild periodontitis - Representativeness of the study population - Lack of information concerning the presence of systemic diseases or medications in both groups. - No blind periodontal examination and sample processing
Kageyama et al, 2017 (30)	NIH quality assessment tool for before-after (Pre-post) study with no control group	50%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - Lack of information on the smoking status of study's participants - No blind periodontal examination and sample processing
Chen *et al.*, 2018 (31)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	33% 50%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - Lack of information on the smoking status of patients in both groups - Lack of information concerning the presence of systemic diseases or medications in both groups. - No blind periodontal examination and sample processing
Damgaard *et al.*, 2019 (28)	NOS for case-control studies	67%	- Representativeness of the study population - Presence of smokers in both groups without adjustment of results according to smoking status - No blind periodontal examination and sample processing
Al-Sabbagh *et al.*, 2012 (32)	NOS for case-control studies	67%	- Definition of controls unclear - Representativeness of the study population - Presence of smokers in the cases group (significant difference with control subjects) without adjustment of results according to smoking status
Sanchez *et al.*, 2013 (33)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	78% 75%	- Representativeness of the study population - No blind periodontal examination and sample processing

Table 1: Qualitative analysis of included studies *(...continued...)*

Authors	Scale for qualitative analysis	Score	Identified risk of bias
Syndergaard *et al.*, 2014 (35)	NOS for case-control studies	44%	- Definition of cases based on unspecified periodontal classification - Definition of controls (BoP and PPD) - Representativeness of the study population - Lack of information on the smoking status of patients in both groups - No blind periodontal examination and sample processing
Morelli *et al.*, 2014 (54)	ROBINS-I	86%	- Representativeness of the study population - Presence of smokers and diabetic patients in both groups without adjustment of results according to smoking status and general health status
Inönü *et al.*, 2020 (34)	NOS for case-control studies	78%	- Representativeness of the study population - No blind periodontal examination and sample processing
Tabari *et al.*, 2013 (36)	NOS for case-control studies	78%	- Representativeness of the study population
Novakovic *et al.*, 2013 (40)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	78% 83%	- Representativeness of the study population - Definition of controls
Dabra *et al.*, 2016 (41)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	44% 42%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear - Representativeness of the study population - No blind periodontal examination and sample processing
Ochanji *et al.*, 2016 (37)	NOS for cross-sectional studies	56%	- Definition of controls unclear - Representativeness of the study population - No blind periodontal examination and sample processing
Mauramo *et al.*, 2017 (42)	NOS for case-control studies	56%	- Lack of information on the systemic condition of patients in both groups (other diseases than diabetes?) or medications (which medication?) - Absence of sample size calculation
Lundmark *et al.*, 2017 (43)	NOS for case-control studies	44%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - In the case group: 1 patient with rheumatoid arthritis, 9 patients with high blood pressure, 3 diabetic patients and 2 patients with cardiovascular disease. And in the control group, 1 patient had a cardiovascular pathology. No adjustment of the results on this factor - Lack of information on the smoking status of patients in different groups - No blind periodontal examination and sample processing
Borges *et al.*, 2018 (38)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	67% 67%	- Representativeness of the study population - Lack of information on the smoking status of patients in different groups - No blind periodontal examination and sample processing

Table 1: Qualitative analysis of included studies *(...continued...)*

Authors	Scale for qualitative analysis	Score	Identified risk of bias
Ansari Moghadam *et al.*, 2019 (39)	NOS for case-control studies NIH quality assessment tool for before-after (Pre-post) study with no control group	56% 42%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear - Representativeness of the study population - No blind periodontal examination and sample processing
Ramseier *et al.*, 2009 (44)	NOS for case-control studies	56%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear and subjects in control group are disparate with patients with gingivitis or healthy periodontium - Presence of smokers in gingivitis patients and cases groups without adjustment of results according to smoking status - No blind periodontal examination and sample processing
Gursoy *et al.*, 2011 (45)	NOS for case-control studies	44%	- Definition of cases based on unspecified periodontal classification - Lack of information on the smoking status of patients in different groups - Lack of information concerning the presence of systemic diseases or medications in both groups. - No blind periodontal examination and sample processing - Absence of sample size calculation
Nomura *et al.*, 2012 (8)	NOS for cross-sectional studies	56%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - Presence of smokers without adjustment of results according to smoking status - No blind periodontal examination and sample processing
Morozumi *et al.*, 2016 (53)	NOS for cross-sectional studies	67%	- Representativeness of the study population - Presence of smokers without adjustment of results according to smoking status - No blind periodontal examination and sample processing
Gursoy *et al.*, 2018 (48)	NOS for cross-sectional studies	56%	- Lack of information about oral status of patients - Lack of information concerning the presence of systemic diseases or medications in both groups. - No blind periodontal examination and sample processing
Sexton *et al.*, 2011 (57)	RoB	71%	- Presence of smokers without adjustment of results according to smoking status
Kinney *et al.*, 2011 (52)	NOS for cross-sectional studies	56%	- Presence of smokers without adjustment of results according to smoking status - No blind periodontal examination and sample processing - Absence of sample size calculation
Lee *et al.*, 2012 (55)	ROBINS-I	86%	- No blind periodontal examination and sample processing
Ebersole *et al.*, 2013 (46)	NOS for case-control studies	56%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - Presence of smokers in the case group without adjustment of results according to smoking status - No blind periodontal examination and sample processing
Ebersole *et al.*, 2015 (47)	NOS for case-control studies	56%	- Definition of cases based on unspecified periodontal classification - Representativeness of the study population - Presence of smokers in the periodontitis group without adjustment of results according to smoking status - No blind periodontal examination and sample processing

Table 1: Qualitative analysis of included studies *(...continued...)*

Authors	Scale for qualitative analysis	Score	Identified risk of bias
Rangbulla *et al.*, 2017 (48)	ROBINS-I	57%	- Definition of cases based on unspecified periodontal classification - Definition of controls unclear - Representativeness of the study population - Lack of information concerning the presence of systemic diseases or medications in cases groups. - No blind periodontal examination and sample processing
Wu *et al.*, 2018 (49)	NOS for case-control studies	78%	- Representativeness of the study population - No blind periodontal examination and sample processing

Notes: NIH: National Institute of Health; NOS: Newcastle-Ottawa Quality Scale; ROBINS-I: Risk of Bias In Non-randomised Studies of Interventions; RoB: The Cochrane Risk of Bias tool for randomised trials

molecules that can be modified during physiological processes (diurnal variation, diet, athlete) or (iii) molecules extracted from crevicular fluid. The 32 selected articles were exclusively human clinical studies, published in English between 2009 and 2020 about periodontal bacteria, inflammation molecules, periodontal tissue degradation molecules present in saliva or their combination as potential diagnostic or prognostic biomarkers of periodontitis. The quality of all selected studies was assessed by the Newcastle-Ottawa Quality Scales for cross-sectional and case-control studies;[22] the National Institute of Health (NIH) quality assessment tool for Before-After (Pre-Post) Studies with No Control Group;[23] the "Risk Of Bias In Non-randomised Studies of Interventions (ROBINS-I)" scale for non-randomised clinical interventional studies[24] and The Cochrane Risk-of-Bias (RoB) tool for randomised trials.[25] Risk of bias was assessed according to the instructions for each scale. The total score was expressed in percentage of absence of potential bias in order to harmonise: (score.100)/maximum score of absence of bias. The scores of the evaluations could reflect a baseline estimate of the quality of each article: 70% and above were recognised as high quality, 30-70% were considered to be of moderate methodological quality, and <30% were classified as low quality (*Table 1*). Details of the scores are provided in *Appendix Tables 1-5*.

Results

Characteristics of the selected studies

For each of the selected studies, the type of study, the type of sought saliva biomarker, the study population, the saliva collection's technique, the main outcomes and the risk of bias were collected (*Table 2*). The analysed studies were case-control studies (n=13) or case-control studies associated with interventional clinical studies (n=7), cross-sectional studies (n=4), cohort studies (n=4) or randomised or non-randomised clinical trials (n=4). The number of patients ranged from 14 to 463 and their ages ranged from 18 to 78 years old. Twenty-one studies included only patients in good general health. Regarding the medical history of the patients, one study accurately detailed the pathologies of the patients in each group, two studies included diabetic patients without indicating whether other pathologies were present and finally eight studies did not provide precise information on the general status of their selected population. Nine studies did not include smokers and nine others did not provide any information on the smoking status of their population. Saliva was collected passively (unstimulated saliva) (n=22) or by chewing paraffin wax (n=8) or gum base (n=2) (stimulated saliva). Salivary molecule analysis techniques included mostly qPCR (n=9) or Next Generation Sequencing (NGS) (n=3) for periodontal bacteria and enzyme immunosorbent assays (ELISA or multiplex) (n=21) for inflammatory molecules and molecules resulting from periodontal tissue breakdown. The qualitative analysis of the studies shows that 75% of them are of moderate quality and 25% of high quality.

Saliva as an aid in the early diagnosis of periodontal disease?

Most of the included studies investigated diagnostic salivary biomarkers (n=25). Twenty were case-control studies more or less associated with clinical trials in diseased patients, three were cross-sectional studies and two were cohort studies with a number of included patients ranged from 27 to 462, from 150 to 170 and from 14 to 463 respectively.[26-50] The potential biases in these studies were related to a lack of information on the medical history and lifestyle (smoking status) of the study population. Six studies focused on periodontal bacteria, four on immune-inflammatory molecules, seven on molecules from periodontal tissue's degradation and eight on a combination of these saliva's compounds as potential diagnostic biomarkers of periodontal disease.

Main findings about periodontal bacteria

The levels of key periodontopathogens in saliva including *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* and *Prevotella intermedia* as well as *Aggregatibacter actinomycetemcomitans,* taken alone or in combination, were associated with the presence of periodontitis compared to the levels found in periodontally healthy individuals.[26-28] For example, Saygun *et al.* (2011) found that the diagnostic sensitivity, i.e., the proportion of true positives in diseased patients for periodontitis, was 89.2% with *P. gingivalis* and *T. forsythia* and 86.5% with *P.*

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
PERIODONTOPATHOGENS						
Hyvärinen *et al.*, 2009 (26)	Case-control study: periodontitis/ no periodontitis	Diagnosis of periodontal status	40-60 years old, minimum 20 teeth Controls (n=81) = all teeth with PPD<4mm – 23% of smokers Cases (n=84) = 14 teeth with PPD≥4mm – unspecified periodontal classification - 57% of smokers	Unstimulated saliva	qPCR assay	- The total bacterial load of the five bacterial species: *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. denticola* and *T. forsythia* is associated with periodontitis with an area under the curve (AUC) of 0.821 (95% 0.758-0.885), p<0.001 - The combination of *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* shows the largest AUC when the different combinations of bacteria are tested: 0.802 (95% 0.734-0.870), p<0.001
Saygun *et al.*, 2011 (27)	Case-Control study: Periodontitis/ gingivitis/ healthy periodontium	Diagnosis of periodontal status	≥18 years old, <6 extracted teeth other than third molars, in good general health with normal salivary flow and without periodontal treatment or antibiotics therapy in the previous 6 months Controls - Healthy periodontium (n=37; mean age 33.1±6.7): PPD≤3mm, absence of BoP, CAL and radiographic bone resorption Cases – unspecified periodontal classification Gingivitis (n=31; mean age 30.9±8.4): presence of BoP on several teeth, PPD≤3mm, absence of CAL and radiographic bone resorption Chronic periodontitis (n=46; mean age 42.7±8.2): at least 9 teeth with PPD=5-7mm and 3 teeth with CAL≥6mm Aggressive periodontitis (n=36; average age 34.5±7.3): at least 14 teeth with CAL≥5mm including 3 teeth other than incisors and 1st molars	Unstimulated saliva	qPCR assay	Diagnostic sensitivity for periodontitis was 89.19% with P. gingivalis (AUC=0,933, p<0,001) and T. forsythia (AUC=0;907, p<0,001) and 86.49% with P. intermedia (AUC=0,874, p<0,001), with specificity ranging from 83.78 to 94.59% when comparing patients with periodontitis (chronic or aggressive) and patients without periodontitis (patients with healthy periodontium or gingivitis).
Yamanaka *et al.*, 2012 (56)	Before-after study: periodontitis patients	Predictive of treatment outcome	35-73 years old, minimum 19 teeth Patients (n=19) with periodontitis (PPD>4mm) in good general health and no periodontal or antibiotics treatment in the previous 6 months - unspecified periodontal classification Periodontitis patients received nonsurgical periodontal treatment Follow-up for 2 years	Stimulated saliva	Pyrosequencing analysis of the 16S rRNA gene	The composition (biodiversity) of salivary bacteria populations was preserved (stable) before and after periodontal treatment (p>0.05) in contrast to the supragingival microbiota (p<0.05).
Salminen *et al.*, 2015 (29)	Case-control study: moderate and severe periodontitis / no and mild periodontitis	Diagnosis of periodontal status	62.9 ± 9.2 years old, 20-29% diabetic 2 groups: Controls: absence of periodontitis or mild periodontitis (n=338): <4 sites with PPD≥4mm - unspecified periodontal classification - 7.9% smokers Cases: moderate to severe periodontitis (n=124): moderate to severe bone resorption and > 4 sites with PPD≥4mm - unspecified periodontal classification - 22.6% smokers	Stimulated saliva	qPCR assay	The presence of moderate to severe periodontitis was assessed more frequently when salivary concentrations of *P. gingivalis* and *T. forsythia* were analyzed in combination: OR= 3.59 (95% CI 1.94-6.63), p<0.001 with sensitivity of 31% and specificity of 89%. This result is adjusted on age, sex, smoking status, presence of diabetes and number of teeth.

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
Kageyama et al, 2017 (30)	Before-after study: periodontitis patients	Diagnosis of periodontal status	35-73 years old 14 subjects diagnosed with chronic periodontitis (PPD>4mm) in good general health and without periodontal treatment or antibiotics in the previous 6 months - unspecified periodontal classification	Stimulated saliva	Pyrosequencing analysis of the 16S rRNA gene	The abundance of 12 periodontopathogens including *P. gingivalis* was positively and linearly correlated: - with the number of sites with PPD≥4mm (r=0.78, p<0.001) - as well as with the presence of these same bacteria in the subgingival plaque before (r=0.61, p=0.02) and after periodontal treatment (r=0.81, p<0.001).
Chen *et al.*, 2018 (31)	Case-control study: periodontitis/ healthy periodontium and before after study: periodontitis patients	Diagnosis of periodontal status and predictive of treatment outcome	Controls: healthy periodontium (n=21) with PPD=3.1±0.42 and BoP=4.8% Cases: chronic periodontitis (n=48) with PPD= 5.7±1.36 and BoP=86% - unspecified periodontal classification Periodontitis patients received nonsurgical periodontal treatment Follow-up for 4 weeks	Unstimulated saliva	Genomic DNA sequencing with Next-Generation Sequencing (NGS)	- significant difference in the composition of microbiota found between saliva and subgingival plaque and between periodontally healthy patients and patients with periodontitis. - Salivary microbiota shows significant variations before and after non-surgical periodontal treatment, particularly about Porphyromonas strains (p=0.02)
Damgaard *et al.*, 2019 (28)	Case-control study: periodontitis/ healthy periodontium	Diagnosis of periodontal status	19-61 years old, in good general health Controls: healthy periodontium (n=25) with 2.3 [1.4-2.7]mm and BoP=4.0% - 13.6% smokers Cases: chronic periodontitis (n=25) with 3.5 [1.8-6.6]mm and BoP=39.4% - 66.6% smokers or aggressive periodontitis (n=31) with 3.6 [2.2-5.3]mm and BoP=56.4% – 1999 American Academy of Periodontology periodontal classification - 34.7% smokers No general disease, no antibiotics treatment in the previous 6 months	Unstimulated saliva	Genomic DNA sequencing with NGS	- Difference in P. gingivalis levels between patients with periodontitis and healthy patients with AUC=0.76 to 0.80, p<0.01 - The presence of P. gingivalis is associated with an increased risk of aggressive periodontitis (RR = 8.1 (95% CI 2.1-31.2), p<0.001) and chronic periodontitis (RR = 6.5 (95% CI 1.6-25.9), p=0.002). - No difference observed between chronic and aggressive periodontitis
MOLECULES OF THE INFLAMMATORY RESPONSE						
Al-Sabbagh *et al.*, 2012 (32)	Case-control study: periodontitis/ no periodontitis	Diagnosis of periodontal status	21-60 years old, in good general health without medication Controls (n=40): BoP<10%, PPD≥5mm in <2% of sites and CAL>2mm in <1% of sites Cases (n=40): Generalized moderate to severe periodontitis with at least 5 sites in each quadrant with PPD≥5mm, CAL≥3mm and BoP - 1999 American Academy of Periodontology periodontal classification Smokers in the cases group only (difference control/case groups: p<0.001)	Unstimulated saliva	Enzyme immunosorbent assays (EIA)	- The average MIP-1α level in subjects with periodontitis was 18 times higher than in healthy subjects (p < 0.0001). - MIP-1α demonstrated the greatest ability to discriminate periodontitis from health: AUC=0.94 with a sensitivity of 94% and a specificity of 92.7%.
Sanchez *et al.*, 2013 (33)	Case-control study: periodontitis/ healthy periodontium and before	Diagnosis of periodontal status	27-61 years old, in good general health and non-smokers 4 groups according to PPD and CAL: Controls (n=15; age 27-41): healthy periodontium Cases: early periodontitis (n=18; age 30-46 years) or moderate	Unstimulated saliva	Enzyme immunosorbent assays (EIA)	- The salivary IL1-β predicted the presence of periodontal disease with a sensitivity of 78% and a specificity of 100% (threshold 212 pg/ml) and AUC>0,90. - PGE2 predicted the presence of periodontal disease with a

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
	-after study: periodontitis patients		periodontitis (n=21; age 33-57 years) or severe periodontitis (n=20; age 32-61 years) - 2007 Page & Eke classification Difference in age between controls and cases groups (periodontitis patients older p<0.05) Periodontitis patients received nonsurgical periodontal treatment Follow-up for 3 months	\		sensitivity of 78% and a specificity of 91% (threshold 121 pg/ml) and AUC>0,90. Age-adjusted results
Syndergaard *et al.*, 2014 (35)	Case-control study: gingivitis/ healthy periodontium	Diagnosis of periodontal status	23-38 years old, minimum 20 teeth, in good general health Controls (n=40): healthy periodontium: PPD≤4mm and CAL≤2mm with BoP<20%. Cases (n=40): gingivitis with PPD≤4mm and CAL≤2mm with BoP≥20% - unspecified periodontal classification Follow-up: 7 to 30 days	Unstimulated saliva	Enzyme immunosorbent assays (EIA and multiplex)	- high levels of PGE2: OR = 35.2 (95% CI: 4.4-282.4) to have gingivitis - high levels of MIP-1α: OR = 8.1 (CI: 1.7-39.3) to have gingivitis
Morelli *et al.*, 2014 (54)	Non-randomized clinical interventional study: induction of a periodontal disease	Prognosis of the disease	170 patients ≥18 years old divided into 5 groups according to BoP and PDD with no periodontal treatment or antibiotics therapy in the previous month and no periodontal drug treatment - 12% smokers Smokers and patients with controlled diabetes have not been excluded. Induction of experimental biofilm overgrowth using stents Follow-up: 7, 14, 21 and 35 days	Unstimulated saliva	Enzyme immunosorbent assays (EIA and multiplex)	Increasing levels of IL-1ra (p=0.0044) and IL-6 (p=0.0093) were the two best predictors of changes in periodontal pockets depths at the peak of gingival inflammation (21 days). No significant salivary predictors of changes in BoP, GI, or CAL was found
Inönü *et al.*, 2020 (34)	Case-control study: Periodontitis/ gingivitis/ healthy periodontium	Diagnosis of periodontal status	≥18 years old, minimum 20 teeth and in good general health, no antibiotics therapy in the previous 6 months, non-smokers Control: Healthy periodontium (n=45): PPD≤3mm, BoP≤10% and absence of radiographic bone resorption Cases: 1999 American Academy of Periodontology periodontal classification - Gingivitis (n=45): BoP>10% and PPD≤3mm and absence of radiographic bone resorption Chronic periodontitis (n=50): CAL≥4mm, PPD≥5mm on more than 4 teeth Generalized aggressive periodontitis (n=40) Difference in age and male/female ratio between controls and cases groups (chronic periodontitis patients older and fewer women p<0.05)	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	Low levels of Del-1 and LFA-1 and high levels of IL-17 combined seem to distinguish periodontitis from gingivitis/or periodontal health: AUC=0.893 (95% CI 0.845-0.94), p<0.001

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
MOLECULES OF TISSUE DEGRADATION						
Tabari *et al.*, 2013 (36)	Case-control study: periodontitis/ healthy periodontium	Diagnosis of periodontal status	22-62 years old, at least 18 teeth, in good general health without antibiotics therapy in the previous 6 months, non-smokers Controls: healthy periodontium (n=25) – BoP<10% and PPD≤3mm Case: chronic periodontitis (n=25) - at least 5 teeth with CAL≥3 mm and PPD>5 mm distributed among at least 2 quadrants - 1999 American Academy of Periodontology periodontal classification	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	- The salivary concentration of RANKL and the RANKL/OPG (osteoprotegerin) ratio were significantly higher in the periodontitis compared to the healthy periodontium group (p=0.004 and p=0.001, respectively). - There were no significant differences in OPG concentration between the two groups (p=0.455).
Novakovic *et al.*, 2013 (40)	Case-control study: periodontitis/ healthy periodontium and before-after study: periodontitis patients	Diagnosis of periodontal status	≥25 years old, general good health, non-smokers Controls (n=21): healthy periodontium with PPD= 2.11±1.67mm Cases (n=42): with moderate chronic periodontitis: bone resorption >30%, PPD>5mm on at least 3 teeth/quadrant - 1999 American Academy of Periodontology periodontal classification - No periodontal treatment in the previous year, nor antibiotics therapy in the previous 3 months. Periodontitis patients received nonsurgical periodontal treatment Follow-up for 2 months	Unstimulated saliva	Colorimetric methods	- Prior to treatment, ALB (albumins) levels were higher in the cases compared to controls patients (p = 0.039), and GXP (glutathione peroxidase) (p = 0.0001) and SOD (superoxide dismutase) (p = 0.021) levels were significantly higher in the cases than in the control group. - After treatment, levels of TAOC (total antioxidant capacity) were higher in the cases compared to control group (p = 0.001), but UA (uric acid) levels were, conversely, higher in the cases than in control group (p = 0.034).
Dabra *et al.*, 2016 (41)	Case-control study: periodontitis/ gingivitis/ healthy periodontium and before-after study: gingivitis and periodontitis patients	Diagnosis of periodontal status	Patients in good general health, non-smokers Controls: healthy periodontium (n=20) Cases: gingivitis (n=20) periodontitis (n=20): at least 4 teeth with CAL≥ 5 mm having moderate bone loss - unspecified periodontal classification Gingivitis and Periodontitis patients received a periodontal treatment by oral hygiene measures + SRP (scaling and root planing)	Stimulated saliva	Spectrometry	- Elevated activity of the enzymes AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma glutamyl transferase), ALP (alkaline phosphatase) and ACP (acidic phosphatase) in the saliva of periodontitis patients compared to healthy periodontal patient group - Decreased activity of the enzymes AST, ALT, GGT, ALP, ACP after non-surgical periodontal treatment.
Ochanji *et al.*, 2016 (37)	Cross-sectional study: a pool of adult patients who visited the dental hospital	Diagnosis of periodontal status	18-75 years old, 158 adults without antibiotics therapy in the previous 3 months and without diseases affecting the periodontium (diabetes, osteoporosis, rheumatoid polyarthritis) - 13.9% smokers Gingivitis (n=77) Mild periodontitis (n=39) Moderate periodontitis (n=24) Severe periodontitis (n=18) - 2007 Page & Eke classification	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	The RANKL/OPG ratio in the diagnosis of periodontitis shows an AUC=0.932 (specificity 95% and specificity 6.2%). This result is adjusted for age and smoking status.
Mauramo *et al.*, 2017 (42)	Cross-sectional study with case-control analysis: adults in the register	Diagnosis of periodontal status	21-58 years old, 258 subjects with 3 diabetic - 14.7% smokers Controls (n=138): healthy periodontium Cases: mild periodontitis (n=5): CAL≥3mm and PPD≥4mm or moderate periodontitis (n=82): CAL≥4mm and PPD≥5mm or severe periodontitis (n=33): CAL≥6mm and PPD≥5mm	Stimulated saliva	immunofluorometric assay	- High levels of MMP-8 are associated with a 2.52 times greater risk of severe periodontitis (95% CI 1.65-4.11). - The salivary MMP-8 levels allow to determine the presence of periodontitis with a specificity of 74% and a sensitivity of 65% - AUC=0.67 (IC95% 0.60-0.74).

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
	of The Blood Transfusion Service SRC Basel		– 2012 Eke *et al.* classification			Results are adjusted for age, gender, smoking status.
Lundmark *et al.*, 2017 (43)	Case-control study: periodontitis/ healthy periodontium	Diagnosis of periodontal status	≥18 years Controls = healthy periodontium (n=39) with PPD<4mm and no gingival inflammation, no BoP Cases = periodontitis (n=37) with PPD≥4mm and ≥6mm, CAL≥5mm and BoP>30 - unspecified periodontal classification	Stimulated saliva	Enzyme immunosorbent assays (multiplex) and DNA sequencing with NGS	Higher levels of salivary and crevicular MMP7 are consistent with overexpression of MMP7 in gingival tissue biopsies from patients with periodontitis.
Borges *et al.*, 2018 (38)	Case-control study: periodontitis/ healthy periodontium and before-after study: periodontitis patients	Diagnosis of periodontal status	At least 14 teeth in good general health and without periodontal treatment in the previous 6 months. Controls (n=9): healthy periodontium, PPD≤3mm and PPD≤3mm%. Cases (n=18): periodontitis stage II grade B, ≥35 years, at - 5 teeth with PPD≥5mm and CAL≥3mm – Chicago, 2017 Periodontitis patients received nonsurgical treatment Follow-up: 2 months	Unstimulated saliva	Enzyme immunosorbent assays (ELISA) and qPCR assays	- Higher expression of RANK-L in the periodontitis group 2.99 pg/mL compared to the control group 1.2 pg/mL (p=0.031) - Higher OPG expression in the periodontitis group before treatment with a 40% reduction after treatment (p=0.0002).
Ansari Moghadam *et al.*, 2019 (39)	Case-control study: periodontitis/ no periodontitis and before-after study: periodontitis patients	Diagnosis of periodontal status and predictive of treatment outcome	≥20 years old, 20 teeth minimum, in good general health, without antibiotics therapy in the previous 3 months, non-smokers Controls (n=14): PPD<3mm and PI≤10% Case (n=13): severe chronic periodontitis with CAL≥5mm on more than 30% of the sites and radiographic bone resorption on at minimum 5 teeth/quadrant - unspecified periodontal classification Periodontitis patients received non-surgical periodontal treatment Follow-up: 4 weeks	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	- The RANKL/OPG ratio is higher in the cases compared to control group (p=0.001). - Significant reduction 4 weeks after periodontal treatment in the RANKL/OPG ratio (p=0.001)
ASSOCIATIONS OF BACTERIA AND MOLECULES OF THE INFLAMMATORY RESPONSE OR TISSUE DEGRADATION						
Ramseier *et al.*, 2009 (44)	Case-control study: periodontitis/ no periodontitis (health and gingivitis)	Diagnosis of periodontal status	20-77 years old, subjects registered with the clinical trials database of the National Institutes of Health (Maryland) minimum 20 teeth 99 patients without periodontal treatment or antibiotics therapy in the previous 6 months and without systemic diseases affecting the periodontium. Exclusion of patients with bone metabolism, autoimmune diseases, osteoporosis and uncontrolled diabetes. Controls: healthy periodontium (n=18) - 0% smokers / gingivitis (n=32): PPD<4mm and CAL<3mm - 19% smokers	Unstimulated saliva	Enzyme immunosorbent assays (ELISA) and qPCR assays	- Levels of MMP-8, MMP-9 and calprotectin increase with increasing periodontal disease destruction. - The combination of MMP-8 with *T. denticola* offers the highest diagnostic accuracy for periodontitis: AUC=0.88; OR= 24.6; 95% CI 5.2-116.5).

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
			Cases: early periodontitis (n=28) - 36% smokers or moderate/severe (n=21): at least 4 sites with bone resorption and CAL>3mm and PPD>4mm - unspecified periodontal classification - 81% smokers Higher smokers in cases groups (p<0.001)			
Gursoy *et al.*, 2011 (45)	Cross-sectional study nested in a cohort study and with case-control analysis: a subsample of the nationally representative population of the "Health 2000 Health Examination Survey"	Diagnosis of periodontal status	Minimum 20 teeth Controls (n=81): healthy periodontium, absence of periodontal pockets Case = Severe periodontitis (n=84): at least 14 teeth with PPD≥4mm - unspecified periodontal classification	Stimulated saliva	Enzyme immunosorbent assays (ELISA) and qPCR assays	The combination of high concentrations (3rd tertile) of *P. gingivalis*, IL-1β and MMP-8 shows an OR = 19.9 (7.11-55.65) and an AUC=0.766 (95% CI 0.694-0.839).
Nomura *et al.*, 2012 (8)	Cross-sectional study nested in a cohort study: periodontitis patients after periodontal treatment	Prognosis of the disease	≥18 years old, in good general health 85 patients who received non-surgical periodontal treatment (SRP) without antimicrobial adjuvants for chronic periodontitis with 2-3 sites with PPD>5mm - unspecified periodontal classification 7.14% smokers among patients without progression and 31.5% among patients with progression of the disease Follow-up: over 18 months	Stimulated saliva	Colorimetric methods and qPCR assays	- *P. gingivalis* rates and the *P. gingivalis*/total bacterial load ratio showed the highest diagnostic probability ratios (specificity 68% and sensitivity 68%, probability 2.13, p=0.002) but could not significantly predict the progression of periodontitis. - The combination of alanine transferase level and *P. gingivalis* ratio showed the highest probability to predict the progression of periodontitis (specificity 96% and sensitivity 40%, probability 11.30, p<0.001).
Morozumi *et al.*, 2016 (53)	Cross-sectional study nested in cohort study: periodontitis patients after periodontal treatment	Prognosis of the disease	≥30 years old, minimum 20 teeth, 4 smokers 124 periodontitis patients in periodontal follow-up after nonsurgical treatment in good general health - 1999 American Academy of Periodontology periodontal classification 62 patients with disease progression 62 patients with controlled periodontitis Follow-up: 2 years	Stimulated saliva	Enzyme immunosorbent assays (ELISA) and qPCR assays	The combination of the *P. gingivalis*/IgG ratio (Immunoglobulin G directed against *P. gingivalis*) has been significantly associated with the progression of periodontitis (sensitivity = 33.9%, specificity = 79%, p=0.001).

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
Gursoy *et al.*, 2018 (48)	Cross-sectional study nested in a cohort study: dentate patients from Parogene study	Diagnosis of periodontal status	463 dentate patients No periodontal classification used	Stimulated saliva	Flow cytometry-based Luminex technique and immunofluorometric assay	Patients with a high cumulative risk (high concentrations of *P. gingivalis*, IL-1β, MMP-8) are 8.9 times more likely to have two sites with a PPD of 4-5mm (OR = 8.9, (2.6-30.4), p<0.001) with AUC=0,725. Results are adjusted for age, gender, smoking status.
ASSOCIATIONS OF INFLAMMATION MOLECULES AND TISSUE DEGRADATION MOLECULES						
Sexton *et al.*, 2011 (57)	Randomized clinical trial before/after treatment: periodontitis patients	Predictive of treatment outcome	≥18 years old periodontitis patients, at least 18 teeth, good general health, PPD≥5mm/CAL≥3mm for 2 teeth in each quadrant - 1999 American Academy of Periodontology periodontal classification - without periodontal treatment in the previous 2 years and antibiotics therapy in the previous 6 months Control group (n=33): patients with oral hygiene measures - 33.3% smokers Test group (n=35): patients with oral hygiene measures + SRP - 22.9% smokers	Unstimulated saliva	Enzyme immunosorbent assays (ELISA and multiplex)	- MMP-8 levels decreased significantly after treatment (p<0.001) only in the SRP group, reflecting the severity of the disease. - OPG, MMP-8 and MIP-1α were significantly reduced in responding patients compared to non-responders in the SRP group (p=0.04, 0.01, 0.05 respectively). Low rates of MMP-8, OPG and MIP-1α after treatment: indication of response to treatment. The best remains MMP-8: AUC ≥0,7, p=0.01.
Kinney *et al.*, 2011 (52)	Cross-sectional study nested in cohort study: a pool of adult patients who visited the Michigan Center for Oral Health Research	Prognosis of the disease	≥18 years old, minimum 20 teeth without periodontal treatment or antibiotics therapy in the previous 3 months and without diseases affecting the periodontium. Healthy periodontium (n=15) - 0% smokers Gingivitis (n=23): PPD<4mm and CAL<3mm - 19% smokers Mild periodontitis (n=24) - 38% smokers Moderate/severe periodontitis (n=17): at least 4 sites with bone resorption and CAL>3mm and PPD>4mm - 80% smokers 1999 American Academy of Periodontology periodontal classification Follow-up: 12 months	Unstimulated saliva	Enzyme immunosorbent assays (ELISA) and qPCR assays	- The high presence of *F. nucleatum, C. rectus* and *P. intermedia,* demonstrated the ability to predict the progression of periodontitis in 82% of individuals. - MMP-8, MMP-9, OPG and IL-1β, present in low concentrations, predicted disease stability in 78% of individuals. - One group of individuals was classified as indeterminate with regard to the clinical course of the disease.
Lee *et al.*, 2012 (55)	Non-randomized clinical interventional study: induction of a periodontal disease	Prognosis of the disease	18-40 years old, minimum 20 teeth 30 patients in good general health, non-smokers with PPD≤4mm and CAL≤2mm, without antibiotics therapy in the previous 6 months Induction of gingivitis: oral hygiene measures are stopped. No periodontal classification used Follow-up: 21 days	Unstimulated saliva	Enzyme immunosorbent assays (ELISA) and DNA-DNA hybridization technique	High levels of IL-6 and MMP-1 at baseline demonstrated the strongest ability to predict induced elevated inflammatory responses (AUC=0.89, 95% CI 1.7-171.7).

Table 2: Summary of the collected data from included studies

Authors	Type of study		Population	Salivary collection technique	Technology of analysis	Main outcomes
Ebersole *et al.*, 2013 (46)	Case-control study: periodontitis/ healthy periodontium	Diagnosis of periodontal status	≥18 years old, 20 teeth minimum Controls (n=30) in good health and with healthy periodontium: BoP<10%, PPD≥5mm (<2% of sites) and CAL>2mm (<1% of sites) - 0% smokers Cases (n=50): chronic periodontitis with 2 teeth in each quadrant with PPD≥5mm, CAL≥3mm, and BOP≥2 for each site - unspecified periodontal classification - 28% smokers	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	The association of IL-1β, IL-6 and MMP-8 discriminated periodontitis from periodontal health: AUC=0.984 (sensitivity 94% and specificity 96%, p=0.001)
Ebersole *et al.*, 2015 (47)	Case-control study: Periodontitis or gingivitis/ healthy periodontium	Diagnosis of periodontal status	≥18 years old, minimum 20 teeth and in good general health Controls (n=65): healthy periodontium with BoP≤10%, PPD≥4mm (<3% of sites), CAL<3mm - 0% smokers Cases: gingivitis (n=43): BoP≥20%, PPD≥4mm (<3% of sites), CAL<3mm - 0% smokers periodontitis (n=101): BoP >10%, PPD≥4mm (>5% of sites), CAL≥2mm - 28% smokers unspecified periodontal classification	Unstimulated saliva	Enzyme immunosorbent assays (Multiplex)	Diagnosis between periodontitis and healthy periodontitis and between gingivitis and periodontitis: combination of IL-1β, IL-6, MMP-8, MIP-1α = sensitivity 78% and specificity 78% (AUC=0.78)
Rangbulla *et al.*, 2017 (49)	Non-randomized clinical interventional study before/after prophylaxis measures	Diagnosis of periodontal status	18-45 years old, minimum 20 teeth, non-smokers and without periodontal or antibiotics therapy or anti-inflammatory treatment in the previous 6 months Controls (n=20) = good general health, healthy periodontium Cases = moderate to severe chronic periodontitis (n=30): PPD≥5mm and CAL≥4mm on at least 3 teeth per quadrant - unspecified periodontal classification All patients received oral prophylaxis measures Folllow-up: 12 weeks	Unstimulated saliva	Enzyme immunosorbent assays (ELISA)	- Higher salivary IgA, IL-1β and MMP-8 concentrations in cases compared to controls (p<0.001) - After implementation of prophylactic measures: reduction in cases of these 3 salivary markers (p<0.001) but remained higher than in controls
Wu *et al.*, 2018 (50)	Case-control study: periodontitis/ healthy periodontium	Diagnosis of periodontal status	20-75 years old, 16 teeth minimum, generally healthy, non-smokers Controls (n=27): healthy periodontium CAL≤2mm without radiographic bone resorption - oral hygiene measure Case (n=30): Generalised chronic periodontitis -1999 American Academy of Periodontology periodontal classification	Unstimulated saliva	Enzyme immunosorbent assays (ELISA and multiplex)	- High levels of MMP-8 and MMP-9 taken individually are associated with a higher risk of periodontitis: OR = 6.0 (95% CI 1.7-22.0), p=0.004. - the combination of IL-1β, IL-1ra and MMP-9: highest AUC=0.853 with high sensitivity 73.3% and specificity 88.9%.

intermedia, with specificity, i.e. the proportion of true negatives in non-diseased patients, ranging from 83.8 to 94.6% when comparing patients with periodontitis and patients without periodontitis (patients with healthy periodontium or gingivitis).[27] The closer the sensitivity and specificity are to 100%, the more effective the biomarkers will be in classifying patients according to their periodontal status.[51] In addition, the ROC areas under the curve (AUC), graphical representation of the relationship between the sensitivity and specificity of a biomarker, were 0.93, 0.91, and 0.87, respectively, and therefore good to excellent.[30] The closer the AUC is to 1, the higher the overall accuracy of the biomarker.[51] In addition, the detection of both *P. gingivalis* and *T. forsythia* in saliva of patients increased by 3.5-fold their risk to develop moderate to severe periodontitis compared to those with mild periodontitis or healthy periodontium. Moreover, a positive correlation between salivary bacterial levels and those of the subgingival plaque was observed and suggests the possibility of differentiating healthy sites from pathological sites through salivary bacterial analysis.[30] In this study of Kageyama *et al.* the authors indeed found that the assessment of the total salivary abundance of 12 bacterial species including *P. gingivalis, T. forsythia, Parvimonas micra* and *F. nucleatum* was better correlated with periodontal health than the relative abundance of one of these species individually studied. However, this observation was not reported by Chen *et al.*[31] Indeed, they found no concordance between salivary microbiota compositions and microbiota composition in the subgingival plaque samples, whether the subjects had healthy or pathological periodontium.[31] It should be emphasised that between the studies conducted by Chen *et al.* (2018) and Kageyama *et al.* (2017) the salivary recovery technique was different: unstimulated saliva and stimulated saliva respectively.[30,31]

Main findings about immune-inflammatory mediators

Research has also focused, over the past ten years, on various mediators of the immuno-inflammatory response. Among them, MIP (macrophage inflammatory protein)-1α, seems to be the most discriminant molecule to detect patients with periodontitis compared to patients with healthy periodontium (94% specificity and 92.7% sensitivity and AUC=0.94). The other immuno-inflammatory molecules studied such as IL (interleukin)-1β, IL-1ra (antagonist receptor), IL-6, IL-17, PGE2 (prostaglandin E2), Del (developmentally regulated endothelial locus)-1 and LFA (Lymphocyte Function Associated)-1) have showed lower sensitivities and specificities.[32-34] Furthermore, patients, with higher salivary PGE2 concentrations, were more susceptible to have gingivitis throughout a clinical examination (OR = 35.2 [95% CI: 4.4-282.4]) than healthy patients with lower salivary PGE2 levels. In this study of Syndergaard *et al.* (2014), similarly, the susceptibility to have gingivitis also increased with the salivary levels of MIP-1α (OR = 8.1 [95% CI: 1.7-39.3]) but was found to be less important than with PGE2.[35]

Main findings about molecules from the degradation of periodontal tissues

Finally, the molecules from the degradation of periodontal tissues were also explored as potential biomarkers of periodontal diagnosis. These molecules, synthetised after the stimulation of the immuno-inflammatory response include metalloproteinases (MMPs) which are enzymes degrading gingival tissue, or molecules involved in osteoclastogenesis. Most studies have identified the RANKL molecule (ligand of the activating receptor of the nuclear factor kB) and more precisely the RANKL/OPG (osteoprotegerin) ratio as a factor that can discriminate healthy periodontium from patients with periodontitis.[33-36] In two studies, the RANKL/OPG ratio was indeed higher in patients with periodontitis than in healthy patients.[36,39] However, Ochanji *et al.* found a good specificity (95%) but a weak sensitivity (6.2%) of this ratio to diagnose a periodontitis and despite a high AUC (0.93).[37] Certain enzymes such as MMP-7, MMP-8 or MMP-9 have also been suggested as possible indicators of periodontitis due to their high salivary levels in periodontitis patients.[40-43] For example, salivary levels of MMP-8 were associated, in the study of Mauramo *et al.*, to 2.52 times more risk to present a severe periodontitis and helped to determine the presence of periodontitis with a specificity of 74%, a sensitivity of 65% and an AUC of 0.67.[42]

Main findings about combination of potential biomarkers

The combination of certain salivary biomarkers previously mentioned seems to improve the accuracy of diagnosis compared to individual biomarkers; and this for the purpose of discrimination between periodontitis and gingival health, as well as for the comparison between gingivitis and periodontitis. Thus, the association of certain matrix metalloproteinases (MMP-8, MMP-9 or MMP-1) with pro-inflammatory cytokines and/or periodontopathogens could be a reliable tool to detect periodontal disease.[41-47] In particular, the combination of IL-6 and MMP-8 demonstrated excellent accuracy [sensitivity 94% and specificity 100% to discriminate healthy periodontitis from periodontitis ($p<0.001$) and 78% and 71% to discriminate gingivitis from periodontitis ($p<0.001$) and AUC greater than 0.75].[46,47]

Saliva as an aid to predict the progression to periodontitis?

Five studies examined the prognostic value of salivary biomarkers in non-treated periodontal disease progression. These were non-randomised clinical trial (n=2), cohort (n=2) or cross-sectional (n=1) studies with 30 to 124 patients. Risks of bias were related to the presence of smokers without adjustment of the results on the smoking status, except in the study of Lee *et al.*[8,52-55]

Main findings

Among the indicators of progression to periodontitis, a high amount of pathogens such as *F. nucleatum, C. rectus* and *P. intermedia* was predictive of disease progression (≥ 2 sites showing a loss of attachment greater than 2 mm over 6 months of follow-up) for 82% of individuals.52 In a 18-month follow-up study, salivary concentrations of *P. gingivalis* and *P. intermedia* in association with concentrations of alanine aminotransferases (ALT), catalytic enzymes, biomarkers of hepatic health, which are involved in the synthesis and degradation of proteins'

amino acids, has been shown to predict the progression of periodontitis with a high specificity of 96% but a low sensitivity of 40% ($p<0.001$).8 The combination of the *P. gingivalis*/IgG (Immunoglobulin G directed against *P. gingivalis*) ratio was also significantly associated with the progression of periodontitis at 2-year follow-up with good specificity (79%) but low sensitivity (33.9%).[53] In addition, MMP-8, MMP-9, osteoprotegerin (OPG) and IL-1β, present in low concentrations, predicted periodontal stability for 78% of individuals who were indeed clinically stable (without disease recurrence) during 12 months follow-up.[52] Furthermore, high levels of IL-6 and IL-1ra was found to be the two best predictors of change in probing depths during the onset of gingival inflammation in patients having stopped all oral hygiene measures.[54] But no other clinical parameters could be predicted by these salivary molecules. In another study, high salivary levels of IL-6 and MMP-1 were strong predictors of the severe gingival inflammation induction in healthy periodontal and non-smokers patients, after 21 days with oral hygiene stop.[55]

Saliva as an aid in predicting response to periodontal treatment?

Four studies examined the relevance of saliva as predictors of periodontal treatment outcomes in 19 to 69 patients diagnosed with periodontitis. In all but one study[39], the risks of bias were mainly due to the absence of adjustment of the results on the smoking status of patients.[31,56-57]

Main findings

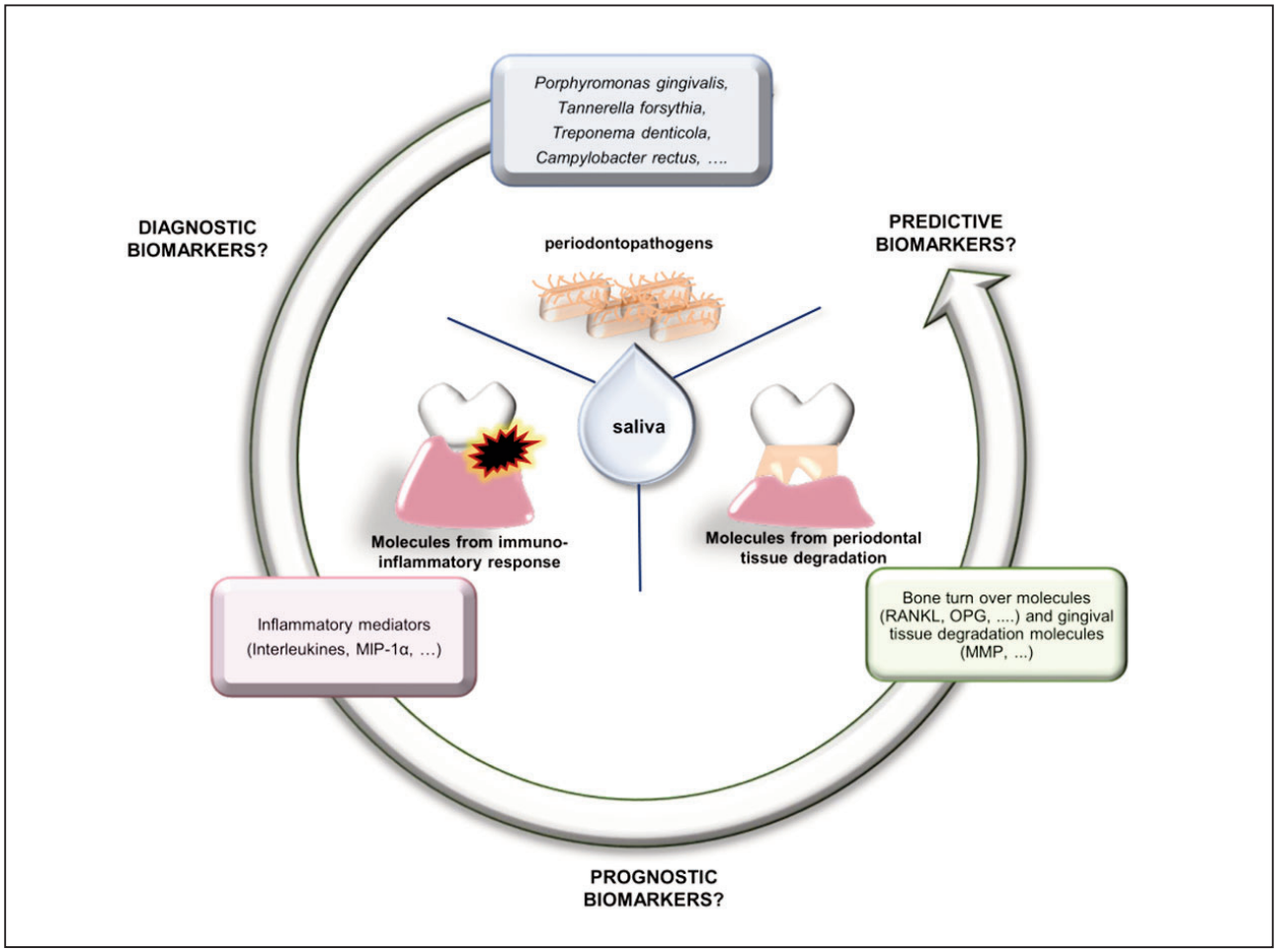
Sexton *et al.*, observed a significant reduction in MMP-8 and MIP-1α in patients who responded well to periodontal therapy compared to non-responders.[57] Salivary concentrations of RANKL/OPG ratio were decreased after periodontal treatment as well as the salivary *P. gingivalis* levels.[31,39] However, in the Yamanaka *et al.* study, no change was observed in the salivary bacterial biodiversity after periodontal treatment as opposed to the supragingival plaque microbiota. It suggested that salivary periodontopathogens could not predict or reflect a positive response to periodontal treatment.[56]

Discussion

In this scoping review, we highlighted associations between various salivary molecules and certain oral bacteria with periodontal clinical parameters[26,27], the progression of the periodontal disease or periodontal treatment outcomes (*Figure 2*).[52] These molecules have been raised as potentially relevant biomarkers, not only for diagnosis but also for prognosis of periodontal disease and treatments.

Among currently available tests on the market, three are based on salivary sample: (i) Periosafe® is a saliva tests based on the immunological assay of MMP-8 which, if positive, means a high risk of periodontitis for the tested patient (Perio Prevention Network); (ii) the MyPerioPath® salivary diagnostic test (MyPerioPath,OralDNA Labs, Brentwood, Tenn) which

Figure 2



identifies specific periodontopathogens that are known to cause periodontal disease and (iii) The MyPerioID® PST® salivary diagnostic test (MyPerioID PST,OralDNA Labs) which identifies individual genetic susceptibility to periodontal disease and increased risk for more severe periodontal infections due to an exaggerated immune response.[58] Overall these salivary tests only provide complementary information to the clinical examination and merely confirm the diagnosis based on clinical and radiologic parameters. They do not allow early diagnosis or anticipation of patients at risk of progression to periodontitis. Indeed, microbiological monitoring of the main periodontopathogens does not seem to be relevant as an early biomarker of periodontitis diagnosis nor as a predictive biomarker of disease progression for all patients.[52,59,60] The collected data in this review are the result of studies using highly variable clinical protocols, inclusion and exclusion criteria, so in this context limited conclusions can be drawn from these studies. Certain immuno-inflammatory molecules have been suggested as biomarkers with an increased relevance of their combination (IL-1β, IL-6 and MIP-1α as well as IL-1β, IL-6, MMP-8 and MIP-1α) or in association with periodontopathogens. In particular, a 2019 systematic review of the literature had also suggested that the combination of the four key biomarkers (IL-1β, IL-6, MMP-8, and MIP-1α) showed promising results for distinguishing between gingivitis and periodontitis.[61] Since periodontal disease is episodic and not all cases of gingivitis progress to periodontitis, identifying biomarkers that can differentiate gingivitis from periodontitis or even predict whether gingivitis could progress to periodontitis could be very useful to clinicians. However, the quality of concerned studies is uneven: type of study, no evaluation of confounding factors [e.g. smoking or systemic pathologies such as diabetes] or use of medications that may affect the quality or quantity of saliva. So, their results should be interpreted with caution. Indeed, in this scoping review, the included studies had a low level of evidence (Grade C) with about three-quarters of the analysed studies presenting moderate risks of bias. Thus, in order to provide patients with individualised care in accordance with their particular needs, a better understanding and analysis of the factors responsible for the transition to periodontitis and the resurgence of bone destruction (that causes the progression of the disease) is required in studies with higher level of evidence.

Beyond the interest in periodontology, salivary analysis has also been proposed for the large-scale evaluation of systemic diseases, such as oral cancers, viral infections (Human Immunodeficiency Virus), autoimmune disorders (Gougerot-Sjögren syndrome, cystic fibrosis), cardiovascular pathologies (atherosclerosis) and endocrine pathologies (diabetes).[12] The molecules of the host's immuno-inflammatory response cannot be specific to periodontal disease, particularly if the patient is suffering from other general diseases. The combination of several salivary and non-salivary biomarkers could help to better take into account the general condition of patients.[52,62] The recognition of risk factors that interact with genetic and epigenetic factors, and the inclusion of cellular and molecular processes and salivary biomarkers could help to define different clinical phenotypes and predict the evolution of all diseases including periodontal disease.[63] It seems a simplistic view to imagine that a single biomarker can provide sufficient information on a disease with pathophysiological mechanisms as complex as that of periodontitis. Future relevant biomarkers useful for the management of patients with periodontitis could result from the multi-biomarkers analysis of multiple origin in a clinical follow-up of the patient. Indeed, it is expected that the combination of (i) genomics (study of DNA sequences) (ii) transcriptomics (study of RNA sequences) (iii) metabolomics (study of the metabolites) (iv) proteomics (study of the proteins) and (v) metagenomics (study of microbiota) will in the future allow the discovery of new biomarkers or the combination of existing biomarkers with new ones and could accelerate personalised and precision medicine and dentistry.[64-66] However, the effect of salivary flow and salivary stimulation as well as the effect of potential physical, chemical and biological aggressions that the oral environment undergoes on a daily basis should be considered in the interpretation and analysis of salivary biomarker concentrations.[67]

The future may also lie with biosensors. Biosensors are a type of wearable sensor used in the continuous measurement of biomarkers in biological fluids, such as saliva, blood and sweat, in order to monitor health and disease status and can assist in the medical diagnosis. Over the past few decades, research has focused on the evaluation of mixtures of multiplexed biosensing, microfluidic sampling, and transport systems integrated with flexible materials and body accessories for portability and simplicity.[68] These devices hold promise for better understanding the correlations between analyte concentrations and feedback to the patient condition. Thus, future biosensors designed to evaluate a broad spectrum of compounds present in biofluids, could help physicians to monitor the control of systemic pathologies, and dentists the oral diseases of patients. Bioinformatics analyses performed using biosensors capable of detecting molecules from complete 'omics' datasets should allow the interpretation of network dynamics of biofluids components and the development of accurate and personalised treatment plans for oral and related systemic conditions.[69] The hope is that such technologies will allow periodontists to identify the molecules capable of predicting the onset and/or evolution of periodontitis.

Conclusion

Current studies have not yet found the biomarker or combination of biomarkers with sufficient sensitivity and specificity to aid in the early diagnosis, prognosis or prediction of the outcomes of periodontal treatment. Future directions should focus on the integration of salivary and non-salivary multi omics data, which should allow to target biomarkers for each patient in order to better understand his or her general condition and medical future with a view to personalised medicine.

Conflict of interest

The authors do not declare any conflict of interest.

Acknowledgments

This article was solicited by the French Society of Periodontology and Oral Implantology (SFPIO). The authors are grateful for the guidance and assistance received during the preparation of the manuscript. We particularly thank Dr Alessandra Blaizot, expert in Public Health, for help in the determination of the qualification of the included studies.

References

1. Armitage GC. The complete periodontal examination. *Periodontol 2000* 2004; **34**: 22-33.

2. Kinney JS, Ramseier CA, Giannobile WV. Oral fluid-based biomarkers of alveolar bone loss in periodontitis. *Ann N Y Acad Sci* 2007; **1098**: 230-251.

3. Du M, Bo T, Kapellas K, Peres MA. Prediction models for the incidence and progression of periodontitis: a systematic review. *J Clin Periodontol* 2018; **45**: 1408-1420.

4. Petsos H, Arendt S, Eickholz P, Nickles K, Dannewitz B. Comparison of two different periodontal risk assessment methods with regard to their agreement: Periodontal Risk assessment versus periodontal risk calculator. *J Clin Periodontol* 2020; **47**: 921-932.

5. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Fickholz P, *et al.* Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions. *J Clin Periodontol* 2018; **45**: S68-S77.

6. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: toward future clinical and diagnostic utilities. *Clin Microbiol Rev.* 2013; **26(4)**:781-791.

7. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *J Prosthet Dent* 2001; **85**: 162-169.

8. Nomura Y, Shimada Y, Hanada N, Numabe Y, Kamoi K, Sato T, *et al.* Salivary biomarkers for predicting the progression of chronic periodontitis. *Arch Oral Biol* 2012; **57**: 413-420.

9. Slaughter YA, Malamud D. Oral diagnostics for the geriatric populations: current status and future prospects. *Dent Clin North Am* 2005; **49**: 445-461.

10. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontol* 2000 2009; **50**: 52-64.

11. Tabak LA. In defense of the oral cavity: the protective role of the salivary secretions. *Pediatr Dent* 2006; **28**: 110-118.

12. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. *J Oral Biol Craniofac Res* 2016; **6**: 66-75.

13. Spielmann N, Wong DT. Saliva: diagnostics and therapeutic perspectives. *Oral Dis* 2011; 1**7**: 345–354.

14. Taba M, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005; **49**: 551-571.

15. Proctor GB. The physiology of salivary secretion. *Periodontol* 2000 2016; **70**: 11–25.

16. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**: 89-95.

17. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J Clin Periodontol* 2011; **38(Suppl.11)**: 85-105.

18. Slots J. Periodontology: Past, present, perspectives. *Periodontol* 2000 2013; **62**: 7-19.

19. Ji S, Choi Y. Point-of-care diagnosis of periodontitis using saliva: technically feasible but still a challenge. *Front Cell Infect Microbiol* 2015; **5**: 65.

20. Taylor JJ. Protein biomarkers of periodontitis in saliva. *ISRN Inflammation* 2014; **2014**: 593151.

21. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, Moher D, Peters MD, Horsley T, Weeks L, Hempel S, *et al.* PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. *Ann Intern Med* 2018; **169**: 467-473.

22. Palmieri V, Colamesta V, La Torre G. Evaluation of methodological quality of studies. *Senses Sci* 2016; **3**: 235-241.

23. Ma LL, Wang YY, Yng ZH, Huang D, Weng H, Zeng XT. Meth odological quality (risk of bias) assessment tools for primary and secondary medical studies: what are they and which is better ? *Mil Med Res* 2020; **7**: 7.

24. Sterne JAC, Hernan MA, Reeves BC, Savovic J, Berkman ND, Viswanathan M, *et al.* ROBINS-I : a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; **355**: i4919.

25. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions.* 2nd Edition. Chichester (UK): John Wiley & Sons, 2019.

26. Hyvärinen K, Laitinen S, Paju S, Hakala A, Suominen-Taipale L, Skurnik M, *et al.* Detection and quantification of five major pathogens by single copy gene-based real-time PCR. *Innate Immun* 2009; **15**: 195-204.

27. Saygun I, Nizam N, Keskiner I, Bal V, Kubar A, Açikel C, *et al.* Salivary infectious agents and periodontal disease status. *J Periodontal Res* 2011; **46**: 325-239.

28. Damgaard C, Danielsen AK, Enevold C, Massarenti L, Nielsen CH, Holmstrup P, *et al.* Porphyromonas gingivalis in saliva associates with chronic and aggressive periodontitis. *J Oral Microbiol* 2019; **11**: 1653123.

29. Salminen A, Kopra KA, Hyvärinen K, Paju S, Mäntylä P, Buhlin K, *et al.* Quantitative PCR analysis of salivary pathogen burden in periodontitis. *Front Cell Infect Microbiol* 2015; **5**: 69.

30. Kageyama S, Takeshita T, Asakawa M, Shibata Y, Takeuchi K, Yamanaka W, *et al.* Relative abundance of total subgingival plaque-specific bacteria in salivary microbiota reflects the overall periodontal condition in patients with periodontitis. PLoS One 2017; 12: e0174782.

31. Chen C, Hemme C, Beleno J, Shi ZJ, Ning D, Qin Y, *et al.* Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. ISME J 2018; 12: 1210-1224.

32. Al-Sabbagh M, Alladah A, Lin Y, Kryscio RJ, Thomas MV, Ebersole JL, *et al.* Bone remodeling-associated salivary biomarker MIP-1β distinguishes periodontal disease from health. J Periodontal Res 2012; 47: 389-395.

33. Sanchez GA, Miozza VA, Delgado A, Busch L. Salivary IL-1β and PGE2 as biomarkers of periodontal status, before and after periodontal treatment. *J Clin Periodontol* 2013; **40**: 1112-1117.

34. Inönü E, Kayis SA, Eskan MA, Hakki SS. Salivary Del-1, IL-17, and LFA-1 levels in periodontal health and disease. *J Periodontal Res* 2020; **55**: 511-518.

35. Syndergaard B, Al-Sabbagh M, Kryscio RJ, Xi J, Ding X, Ebersole JL, *et al.* Salivary biomarkers associated with gingivitis and response to therapy. *J Periodontol* 2014; **85**: e295-303.

36. Tabari ZA, Azadmehr A, Tabrizi MA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. *J Periodontal Implant Sci* 2013; **43**: 227-232.

37. Ochanji AA, Matu NK, Mulli TK. Association of salivary RANKL and osteoprotegerin levels with periodontal health. *Clin Exp Dent Res* 2017; **3**: 45-50.

38. Borges CD, Ricoldi MS, Messora MR, Palioto DB, Souza SLS, Novaes Junior AB, *et al.* Clinical attachment loss and molecular profile of inflamed sites before treatment. *J Appl Oral Sci* 2019; **27**: e20180671.

39. Ansari Moghadam S, Sarani S, Alijani E, Ansari Moghadam A. The effect of Phase 1 periodontal treatment on the salivary RANKL/OPG ratio in severe chronic periodontitis. *Clin Cosmet Investig Dent* 2019; **11**: 251-257.

40. Novakovic N, Todorovic T, Rakic M, Milinkovic I, Dozic I, Jankovic S, *et al.* Salivary antioxidants as periodontal biomarkers in evaluation of tissue status and treatment outcome. *J Periodontal Res* 2014; **49**: 129-136.

41. Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. *J Indian Soc Periodontol* 2012; **16**: 358-364.

42. Mauramo M, Ramseier AM, Mauramo E, Buser A, Tervahartiala T, Sorsa T, *et al.* Associations of oral fluid MMP-8 with periodontitis in Swiss adult subjects. *Oral Dis* 2018; **24**: 449-455.

43. Lundmark A, Johannsen G, Erikson K, Kats A, Jansson L, Tervahartiala T, *et al.* Mucin 4 and matrix metalloproteinase 7 as novel salivary biomarkers for periodontitis. *J Clin Periodontol* 2017; **44**: 247-254.

44. Ramseier CA, Kinney JS, Herr AE, Braun T, Sugai JV, Shelburne CA, *et al.* Identification of pathogen and host-response markers correlated with periodontal disease. *J Periodontol* 2009; **80**: 436-446.

45. Gursoy UK, Könönen E, Pussinen PJ, Tervahartiala T, Hyvärinen K, Suominen AL, *et al.* Use of host- and bacteria-derived salivary markers in detection of periodontitis: a cumulative approach. *Dis Markers* 2011; **30**: 299-305.

46. Ebersole JL, Shuster JL, Stevens J, Dawson D. 3rd, Kryscio RJ, Lin Y, *et al.* Patterns of salivary analytes provide diagnostic capacity for distinguishing chronic adult periodontitis from health. *J Clin Immunol* 2013; **33**: 271-279.

47. Ebersole JL, Nagarajan R, Akers D, Miller CS. Targeted salivary biomarkers for discrimination of periodontal health and disease(s). *Front Cell Infect Microbiol* 2015; **5**: 62.

48. Gursoy UK, Pussinen PJ, Salomaa V, Syejäläinen S, Könönen E. Cumulative use of salivary markers with an adaptive design improves detection of periodontal disease over fixed biomarker thresholds. *Acta Odontol Scand* 2018; **76**: 493-496.

49. Rangbulla V, Nirola A, Gupta M, Batra P, Gupta M. Salivary IgA, interleukin-1β and MMP-8 as salivary biomarkers in chronic periodontitis patients. *Clin J Dent Res* 2017; **20**: 43-51.

50. Wu YC, Ning L, Tu YK, Huang CP, Huang NT, Chen YF, *et al.* Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population. *J Formos Med Assoc* 2018; **117**: 841-848.

51. Delacour H, Servonnet A, Perrot A, Vigezzi JF, Ramirez JM. ROC (receiver operating characteristic) curve: Principles and application in biology. *Ann Biol Clin* 2005; **63**: 145-154.

52. Kinney JS, Morelli T, Braun T, Ramseier CA, Herr AE, Sugai JV, *et al.* Saliva/pathogen biomarker signatures and periodontal disease progression. *J Dent Res* 2011; **90**: 752–758.

53. Morozumi T, Nakagawa T, Nomura Y, Sugaya T, Kawanami M, Suzuki F, *et al.* Salivary pathogen and serum antibody to assess the progression of chronic periodontitis: a 24-mo prospective multicenter cohort study. *J Periodontal Res* 2016; **51**: 768-778.

54. Morelli T, Stella M, Barros SP, Marchesan JT, Moss KL, Kim SJ, *et al.* Salivary biomarkers in a biofilm overgrowth model. *J Periodontol* 2014; **85**: 1770-1778.

55. Lee A, Ghaname CB, Braun TM, Sugai JV, Teles RP, Loesche WJ, *et al.* Bacterial and salivary biomarkers predict the gingival inflammatory profile. *J Periodontol* 2012; **83**: 79-89.

56. Yamanaka W, Takeshita T, Shibata Y, Matsuo K, Eshima N, Yokoyama T, *et al.* Compositional stability of a salivary bacterial population against supragingival microbiota shift following periodontal therapy. *PLoS One* 2012; **7**: e42806.

57. Sexton WM, Lin Y, Kryscio RJ, Dawson DR. 3rd, Ebersole JL, Miller CS. Salivary biomarkers of periodontal disease in response to treatment. *J Clin Periodontol* 2011; **38**: 434-441.

58. Genco RJ. Salivary diagnostic tests. *JADA* 2012; **143**: 3S-5S.

59. Listgarten MA, Loomer PM. Microbial identification in the management of periodontal diseases. A systematic review. *Ann Periodontol* 2003; **8**: 182-192.

60. Zhang L, Henson, BS, Camargo PM, Wong DT. The clinical value of salivary biomarkers for periodontal disease. *Periodontol* 2000 2009; **51**: 25-37.

61. Kc S, Wang XZ, Gallagher JE. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: systematic review. J Clin Periodontol 2020; 47: 289-308.

62. Korte DL, Kinney J. Personalized medicine: an update of salivary biomarkers for periodontal diseases. *Periodontol* 2000 2016; **70**: 26-37.

63. Offenbacher S, Barros SP, Singer RE, Moss K, Williams RC, Beck JD. Periodontal disease at the biofilm-gingival interface. *J Periodontol* 2007; **78**: 1911-1925.

64. Hartenbach FARR, Velasquez E, Nogueira FCS, Domont GB, Ferreira E, Colombo APV. Proteomic analysis of whole saliva in chronic periodontitis. *J Proteomics* 2020; **213**: 103602.

65. Lelapi N, Andreucci M, Licastro N, Faga T, Grande R, Buffone G, *et al.* Precision medicine and precision nursing: the era of biomarkers and precision health. *Int J Gen Med* 2020; **13**: 1705-1711.

66. Granata I, Manzo M, Kusumastuti A, Guarracino MR. Learning from metabolic networks: current trends and future direction for precision medicine. *Curr Med Chem* 2020 doi: 10.2174/0929867328666 2012 17103148

67. Vila T, Rizk AM, Sultan AS, Jabra-Rizk MA. The power of saliva: Antimicrobial and beyond. *PLoS Pathog* 2019; **15**: e1008058.

68. Sharma A, Badea M, Tiwari S, Marty JL. Wearable biosensors : an alternative and practical approach in healthcare and disease monitoring. *Molecules* 2021; **26**: 748.

69. Steigmann L, Maekawa S, Sima C, Travan S, Wang CW, Giannobile WV. Biosensor and Lab-on-a-chip biomarker-identifying technologies for oral and periodontal diseases. *Front Pharmacol* 2020; **11**: 588480.

Appendix

Appendix Table 1: Methodological quality evaluation of case-control studies according to the Newcastle-ottawa scale (NOS) for case-control studies

Controls cases studies	**SELECTION** - Definition of cases, - Representativeness of cases, - Selection of controls, - Definition of controls	**COMPARATIBILITY** - Comparability of cases and controls based on the design or analysis	**EXPOSURE** - Ascertainment of exposure, - Non-response rate	**TOTAL** (Absence of bias)
	4 points	**2 points**	**3 points**	**9 points**
Hyvärinen *et al.* 2009 (26)	2	1	2	**5**
Saygun *et al.* 2011 (27)	4	1	2	**7**
Salminen *et al.* 2015 (29)	1	1	2	**4**
Chen *et al.* 2018 (30)	0	1	2	**3**
Damgaard *et al.* 2019 (28)	3	1	2	**6**
Al-Sabbagh *et al.* 2012 (32)	2	1	3	**6**
Sanchez *et al.* 2013 (33)	3	2	2	**7**
Syndergaard *et al.* 2014 (35)	1	1	2	**4**
Inönü *et al.* 2020 (34)	3	2	2	**7**
Tabari *et al.* 2013 (36)	3	2	2	**7**
Novakovic *et al.* 2014 (40)	3	2	2	**7**
Dabra *et al.* 2016 (41)	1	1	2	**4**
Mauramo *et al.* 2017 (42)	2	1	2	**5**
Lundmark *et al.* 2019 (43)	1	1	2	**4**
Borges *et al.* 2018 (38)	3	1	2	**6**
Ansari Maghadam *et al.* 2019 (39)	1	2	2	**5**
Ramseier *et al.* 2009 (44)	2	1	2	**5**
Gursoy *et al.* 2011 (45)	2	1	1	**4**
Ebersole *et al.* 2013 (46)	2	1	2	**5**
Ebersole *et al.* 2015 (47)	2	1	2	**5**
Wu *et al.* 2018 (50)	3	2	2	**7**

Appendix Table 2: Methodological quality evaluation of cross-sectional studies according to the Newcastle-ottawa scale (NOS) for cross-sectional studies

Cross-sectional studies	SELECTION - Representativeness of sample - Sample size - Non-respondents - Ascertainsment of exposure	COMPARATIBILITY - Comparability of the groups based on study design or analysis	EXPOSURE - Assessment of outcome - Statistical test	TOTAL (Absence of bias)
	5 points	**2 points**	**2 points**	**9 points**
Ochanji *et al.* 2016 (37)	2	2	1	**5**
Nomura *et al.* 2012 (8)	2	1	2	**5**
Morozumi *et al.* 2016 (53)	3	1	2	**6**
Gursoy *et al.* 2018 (48)	2	1	2	**5**
Kinney *et al.*2011 (52)	3	1	1	**5**

Appendix Table 3: Methodological quality evaluation of before-after studies accordig to the National Institute of Health (NIH) quality assessment tool for Before-After (Pre-Post) Studies with No Control Group

Before-after studies	SELECTION - Study question - Eligibility criteria - Representativeness of study population - All eligible participants enrolled	SAMPLE SIZE	INTERVENTION	OUTCOMES - Description - Blind - Multiple outcomes measures	FOLLOW-UP	STATISTICAL ANALYSIS	GROUP-LEVEL INTERVENTION	TOTAL (Absence of bias)
	4 points	**1 pt**	**1 pt**	**3 points**	**1 pt**	**1 pt**	**1 pt**	**12 points**
Yamanaka *et al.* 2012 (56)	2	0	0	2	1	0	1	**6**
Kageyama *et al.* 2017 (30)	2	1	0	1	0	1	1	**6**
Chen *et al.* 2018 (31)	2	0	0	2	0	1	1	**6**
Sanchez *et al.* 2013 (33)	3	0	1	2	1	1	1	**9**
Novakovic *et al.* 2013 (40)	3	1	1	2	1	1	1	**10**
Dabra *et al.* 2016 (41)	2	0	0	1	0	1	1	**5**
Borges *et al.* 2018 (38)	2	0	1	2	1	1	1	**8**
Ansari Mogadham *et al.* 2019 (39)	1	0	0	2	0	1	1	**5**

Appendix Table 4: Methodological quality evaluation of non-randomised clinical trials according to the Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I)

Non-randomised clinical trial	Bias due to confounding	Bias in selection participants	Bias in class-ification of intervention	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measure-ment of outcomes	Bias in selection of the reported result	TOTAL (Risk of bias)
Morelli *et al.* 2014 (54)	1	0	0	0	0	0	0	**1**
Lee *et al.* 2012 (55)	0	0	0	0	0	1	0	**1**
Rangbulla *et al.* 2017 (49)	1	1	0	0	0	1	0	**3**

Appendix Table 5: Methodological quality evaluation of randomised clinical trials according to the Cochrane risk of bias tool (RoB)

Randomised clinical trial	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome	Incomplete outcome data	Selective reporting	Other bias	TOTAL (Risk of bias)
Sexton *et al.* 2011 (57)	0	0	0	1	0	0	1	**2**