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REVIEW

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Accuracy of periodontitis diagnosis obtained using multiple molecular biomarkers in oral fluids: A systematic review and meta-analysis

T. Blanco-Pintos[1] | A. Regueira-Iglesias[1] | I. Seijo-Porto[1] | C. Balsa-Castro[1] | P. Castelo-Baz[1] | L. Nibali[2] | I. Tomás[1]

[1]Oral Sciences Research Group, Special Needs Unit, Department of Surgery and Medical-Surgical Specialties, School of Medicine and Dentistry, Universidade de Santiago de Compostela, Health Research Institute of Santiago (IDIS), Santiago de Compostela, Spain

[2]Periodontology Unit, Centre for Host-Microbiome Interactions, Dental Institute, King's College London, London, UK

Correspondence
I. Tomás, Oral Sciences Research Group, Special Needs Unit, Department of Surgery and Medical-Surgical Specialties, School of Medicine and Dentistry, Universidade de Santiago de Compostela, 15872 Santiago de Compostela, Spain.
Email: inmaculada.tomas@usc.es

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Abstract

Aim: To determine the accuracy of biomarker combinations in gingival crevicular fluid (GCF) and saliva through meta-analysis to diagnose periodontitis in systemically healthy subjects.

Methods: Studies on combining two or more biomarkers providing a binary classification table, sensitivity/specificity values or group sizes in subjects diagnosed with periodontitis were included. The search was performed in August 2022 through PUBMED, EMBASE, Cochrane, LILACS, SCOPUS and Web of Science. The methodological quality of the articles selected was evaluated using the QUADAS-2 checklist. Hierarchical summary receiver operating characteristic modelling was employed to perform the meta-analyses (CRD42020175021).

Results: Twenty-one combinations in GCF and 47 in saliva were evaluated. Meta-analyses were possible for six salivary combinations (median sensitivity/specificity values): IL-6 with MMP-8 (86.2%/80.5%); IL-1β with IL-6 (83.0%/83.7%); IL-1β with MMP-8 (82.7%/80.8%); MIP-1α with MMP-8 (71.0%/75.6%); IL-1β, IL-6 and MMP-8 (81.8%/84.3%); and IL-1β, IL-6, MIP-1α and MMP-8 (76.6%/79.7%).

Conclusions: Two-biomarker combinations in oral fluids show high diagnostic accuracy for periodontitis, which is not substantially improved by incorporating more biomarkers. In saliva, the dual combinations of IL-1β, IL-6 and MMP-8 have an excellent ability to detect periodontitis and a good capacity to detect non-periodontitis. Because of the limited number of biomarker combinations evaluated, further research is required to corroborate these observations.

KEYWORDS
diagnostic accuracy, meta-analysis, molecular biomarkers, oral fluids, periodontitis

Clinical Relevance

Scientific rationale for study: It is recognized that biomarkers may be essential in the early diagnosis of periodontitis. Because of the disease's complexity, combining more than one biomarker may be appropriate.

Principal findings: In saliva, IL-1β with MMP-8, IL-1β with IL-6 or IL-6 with MMP-8 as a first-line screening tool would correctly identify 78%–81% of periodontitis patients and 85%–88% of non-periodontitis subjects. These results did not change by combining these three biomarkers.
Practical implications: Dual associations of IL-1β, IL-6 and MMP-8 in saliva show their clinical utility in diagnosing periodontitis.

1 | INTRODUCTION

Periodontitis is one of the most prevalent chronic diseases (Kassebaum et al., 2014). In 2017, the age-standardized prevalence of severe periodontitis was 9.8%, representing 796 million people affected by this disease worldwide (Bernabe et al., 2020). These numbers are likely to continue to increase as the population ages (Tonetti et al., 2017).

This disease reduces oral-health-related quality of life (Graziani & Tsakos, 2020), and it has been linked to more than 50 systemic conditions (Tsuchida, 2020), including diabetes, cardiovascular diseases (Liccardo et al., 2019) and dementia (Asher et al., 2022). Therefore, an accurate and prompt diagnosis of the periodontal condition is essential, because bone and soft-tissue loss may lead to aesthetic, functional and systemic sequelae (Papapanou et al., 2018).

Clinical measures enable clinicians to assess the current extent and severity of periodontitis and past tissue destruction, but they do not provide reliable data about the biological activity or future course (Tsuchida, 2020). Furthermore, recording clinical measures is subjective, as it is examiner-dependent (Preianò et al., 2020). Considering these limitations, the current classification of Periodontal and Peri-implant Diseases and Conditions has highlighted the potential use of biomarkers to improve the early diagnosis of periodontitis (Tonetti et al., 2018).

The destruction of the periodontium is caused by a microbial dysbiosis that overactivates the host's immunological response (Hajishengallis, 2014; Pan et al., 2019). This alters the expression of several proteins or metabolites (Tsuchida, 2020), and molecular biomarker profiles specific to each periodontal condition in oral fluids have been demonstrated (Almehmadi & Alghamdi, 2018; Baima et al., 2021; Chen et al., 2019; de Morais et al., 2018; Di Lenardo et al., 2019). This has prompted the publication of many systematic reviews/meta-analyses on the diagnostic accuracy of single molecular biomarkers in the gingival crevicular fluid (GCF) (Arias-Bujanda et al., 2019) and saliva (Arias-Bujanda et al., 2020; de Lima et al., 2016).

However, it has been recognized that, because of the complexity of the disease and the combined action of different inflammatory/tissue destruction components, it may be appropriate to combine more than one biomarker (Almehmadi & Alghamdi, 2018; Bibi et al., 2021; Zhang et al., 2009). To our knowledge, only one systematic review has evaluated the accuracy of biomarker combinations in saliva to diagnose periodontitis (Sukriti et al., 2020). However, no meta-analysis has been published in both oral fluids (GCF and saliva), so further evidence is required to advance the topic.

Therefore, the objective of the present systematic review/meta-analysis was to determine the diagnostic accuracy for periodontitis derived from a combination of biomarkers, with at least one molecular biomarker detected in GCF or saliva in systemically healthy subjects.

2 | METHODS

This systematic review was conducted according to the PRISMA-DTA (Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy) statement (McInnes et al., 2018) and the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy, version 1.0.0 (Deeks & Bossuyt, 2013).

The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) and was assigned the number CRD42020175021. A detailed description of the methods is given in Appendix S1.1.

2.1 | Focused question

The formulated focused question was as follows: 'In systemically healthy subjects, does the expression of a combination of molecular biomarkers in oral fluids (GCF or saliva) enable professionals to make periodontitis diagnoses comparable to those produced with clinical parameters?'

2.2 | Inclusion criteria

2.2.1 | Types of accuracy studies

The review encompassed all investigations that evaluated a combination of at least two molecular biomarkers in GCF, saliva or both (index test) that provided a diagnosis in individuals with periodontitis detected by conventional clinical parameters (reference standard). Eligible study designs were cross-sectional or longitudinal with diagnostic accuracy evaluation. Studies were excluded if they did not contain a binary classification table (number of true positives, true negatives, false positives, and false negatives) or adequate information to calculate it (sample size and sensitivity/specificity values).

2.2.2 | Definitions of target and control conditions

Participants were patients without a specific diagnosis of a systemic disease but had a clinical periodontal diagnosis. Target and control

conditions were defined at the patient (GCF and saliva) or site level (only GCF). At the patient level, the target condition was periodontitis at any stage/degree of severity and extent. Patients diagnosed with periodontal health and gingivitis were included as controls. At the site level, the target condition consisted of periodontitis sites according to the criteria considered by the authors, and the control condition was healthy, gingivitis or <2 mm clinical attachment loss (CAL) sites.

2.2.3 | Index test(s) and reference standard

An index test was defined as two or more objectively quantifiable biomarkers in GCF or saliva, at least one of them being a molecular biomarker, which were mathematically combined in a predictive model.

The reference standard was defined as the best method for establishing the presence/absence of the target condition (Cohen et al., 2016). This required diagnosing the periodontal condition based on only clinical (probing pocket depth [PPD] or CAL) or clinical and radiographic parameters (bone loss [BL]). The definition of periodontal status was based either on previously established criteria (Armitage, 1999; Caton et al., 2018) or on the criteria reported by the author(s). Conversely, research that did not specify any reference standard or where periodontal status was not assessed using at least one clinical parameter (PPD or CAL) was excluded.

2.3 | Search methods for the identification of studies

The searches were conducted using the electronic databases PUBMED (MEDLINE), EMBASE, Cochrane Central Register of Controlled Trials and Trial Protocols, LILACS, SCOPUS and Web of Science.

The search strategy employed in August 2022 adopted the recommendations of the Cochrane Group for Systematic Reviews of Diagnostic Test Accuracy (Deeks & Bossuyt, 2013), which is detailed in Appendix S1.2.

2.3.1 | Selection of studies applying a dual process: Data mining and manual methods

R software (version 4.2.2) and packages downloaded from the Comprehensive R Archive Network Team (2022) were employed for the management of the data detected through the searches (R Core Team, 2022).

Computational analysis of the abstracts was performed as described in Appendices S1.1 and S1.3 to guarantee research reproducibility (Norman et al., 2018), using the tm (version 0.7-10) and NLP packages (version 0.2-1) (Feinerer et al., 2008; Feinerer & Hornik, 2022; Hornik, 2020). The automated data-mining process had been previously validated, as explained before (Arias-Bujanda et al., 2019, 2020).

The manual evaluation of articles with multiple published identifiers (PMIDs), those with a single PMID without abstract or those without PMID, besides full-text candidate papers, was performed by three independent reviewers (T.B.P., A.R.I. and I.S.P.), who recorded the reasons for exclusion. All disagreements were resolved by discussion and consultation with a fourth reviewer (I.T.). Agreement between reviewers was assessed using the percentage of agreement and kappa statistic.

2.3.2 | Data extraction and management

Data from selected studies were independently extracted in triplicate by three authors (T.B.P., A.R.I. and I.S.P.) using a standardized data collection form. The information recorded from each paper was as follows: type of study, number and type of control and target conditions, patient characteristics, reference standard characteristics, GCF and saliva samples characteristics, type of biomarkers, detection technique employed and diagnostic accuracy results.

2.4 | Assessment of methodological quality

Two reviewers (C.B.C. and P.C.B.) independently assessed the methodological quality of the studies included using the critical review checklist described in the revised Quality Assessment of Diagnostic Studies (QUADAS-2) (Whiting et al., 2011). According to our previously published reviews (Arias-Bujanda et al., 2019, 2020), the QUADAS-2 checklist was modified, removing the first question of the 'index test' domain (Appendix S2.1).

2.5 | Qualitative analysis

The unit of analysis was each binary classification table of a combination of at least two biomarkers. The diagnostic accuracy of a biomarker combination was assessed by measuring the biomarkers' capacity to detect the presence or absence of the target condition.

Precision estimates were expressed as sensitivity and specificity values and their corresponding 95% confidence intervals for each biomarker combination (index test) and visualized using forest plots. Other accuracy measures, including accuracy (ACC), positive predictive value (PPV), negative predictive value (NPV), positive likelihood value (LR+), negative likelihood value (LR−), diagnostic odds ratio (DOR) and Youden's index (Youden, 1950) were calculated using the data extracted from each article. The diagnostic accuracy parameters were interpreted using the previously established guidelines defined in Appendix S3.1.

2.6 | Quantitative analysis

Meta-analysis was performed when at least three diagnostic classification tables were calculated for a GCF or salivary biomarker

combination in at least two studies. Accuracy studies were included regardless of the threshold value. Hierarchical summary receiver operating characteristic (HSROC) modelling was used to conduct the meta-analysis using the HSROC package (version 2.1.9) (Schiller & Dendukuri, 2019).

To produce direct evidence of the effectiveness of GCF and salivary biomarker combinations subjected to meta-analyses (Leeflang & Kraaijpoel, 2018), we considered the summary accuracy data obtained using natural frequencies based on a hypothetical cohort of 1000 patients (Whiting et al., 2018). These frequencies were then converted into percentage values regarding summary estimates of sensitivity, specificity and different prevalences of periodontitis.

3 | RESULTS

3.1 | Study selection

We conducted 353 searches per database, which retrieved 16,716 articles, of which computational data-mining techniques deleted 89.7%, and the remaining 10.3% were assessed manually. Then, 438 papers were selected for full-text assessment, besides 13 that were detected through citation searching. Subsequently, 428 articles were excluded for different reasons (Appendix S4). Finally, 10 publications in GCF and 13 in saliva—including 21 contingency tables in GCF and 70 in saliva—were qualitatively evaluated (Altıngöz et al., 2021; Arias-Bujanda et al., 2018; Baeza et al., 2016; Baliban et al., 2013; Beighton et al., 1990, 1992; Ebersole et al., 2013, 2015; Ferrando et al., 2005; Görgülü & Doğan, 2022; Grant et al., 2022; Hanioka et al., 2005; Huang et al., 2020; Inönü et al., 2020; Kim et al., 2021; Kitamura et al., 1991; Nagarajan et al., 2015; Nakashima et al., 1996; Ochanji et al., 2017; Rzeznik et al., 2017; Tomás et al., 2017; Wu et al., 2018; Zhang et al., 2021). Appendix S5 shows this flow-chart. The percentage of agreement and kappa scores for title and abstract screening were 96.1% and 0.90, respectively, and for full-text evaluation they were 99.3% and 0.96.

3.2 | Characteristics of diagnostic accuracy studies in GCF and saliva

Twenty-one different combinations of biomarkers in GCF and 47 in saliva were extracted. The combination of two biomarkers was the most frequent (57.1% in GCF and 52.9% in saliva), whereas 47.1% and 42.9% were formed by 3–5 and 5–10 biomarkers, respectively. Regarding the type of biomarkers, 85.7% and 80.0% of the classifications in GCF and saliva comprised one or more inflammatory mediators. Protein groups comprising nine or more GCF biomarkers are specified in Appendix S6.

The mean age of patients with the target condition was above 40 years in 70% of GCF studies and 76.9% in saliva, whereas that for the control subjects were between 30 and 45 years in 70% of GCF studies and between 20 and 40 years in 61.5% in saliva.

The most frequent target condition in GCF was chronic periodontitis at different stages (76.2%), with Armitage's classification (Armitage, 1999) being the most used (61.9%), followed by sites with CAL ≥2 mm in chronic periodontitis patients (14.3%). In saliva, it was periodontitis at different stages (74.3%), being the new classification (Caton et al., 2018) the most used (51.4%), especially at stage III (45.7%).

In GCF, the control condition most employed was healthy periodontium (71.4%), based on bleeding on probing (BOP) <25%, absence of PPD ≥4 mm and radiographic BL in 42.9% of the classifications, followed by sites with no CAL in chronic periodontitis patients (14.3%). In saliva, it was periodontal health (62.9%), followed by gingivitis (24.3%), which were defined as BOP <10% (periodontal health) or ≥20% (gingivitis), CAL <2 mm and PPD <4 mm in 35.7% of the classifications.

In 50% of the GCF articles, the participants' tobacco use was not specified; 30% included non-smokers and smokers, and non-smokers formed 20%. In saliva, 46.2% of the articles included smokers and non-smokers, the same percentage selected only non-smokers, while one paper did not provide this information.

The technique most employed for biomarker detection in GCF was multiparametric cytometry (42.9%), whereas in saliva it was enzyme-linked immunosorbent assay (ELISA) (64.3%), followed by multiparametric cytometry (42.9%). This information is detailed in Table 1 and summarized in Appendix S7.

3.3 | Quality assessment of diagnostic accuracy studies in GCF and saliva

The patient selection domain was considered at high risk of bias in ≥90% of the articles in GCF and saliva, mainly due to the employment of case–control designs (80% in GCF and 92.3% in saliva). The index test domain was categorized as having a high risk of bias in the 23 papers because none applied pre-specified thresholds. However, the reference standard domain was judged as having a low risk of bias in ≥92% of the studies (in both GCF and saliva). The interval between the reference standard and the index test was adequate in 70% of the GCF articles (low risk of bias), but 30% did not specify this period (unclear risk of bias); in saliva, these values were 23.1% and 76.9%, respectively. The applicability of all domains was considered at low risk of bias in all papers, except one on saliva that employed as reference standard the Community Periodontal Index of Treatment Needs (CPITN) system, which was judged as unclear risk (Figure 1, Appendices S2.2 and S2.3).

Considering the sample size as a quality indicator, most of the target and control condition classifications in GCF were composed of ≤30 subjects (57.1% and 71.4%, respectively), but considering the number of samples, 57.1% evaluated ≥31 samples. In saliva, 64.3% of target condition classifications comprised ≥31 participants, while 61.4% of control condition classifications were formed by ≤30 subjects (Table 1, Appendix S7).

TABLE 1 Main characteristics of the articles included in the present systematic review that analysed combinations of biomarkers in gingival crevicular fluid (GCF) and saliva ($N = 23$ articles).

Main characteristics of the articles that analysed combinations of GCF biomarkers ($N = 10$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of GCF sample (storage)	Biomarkers evaluated (technique; detection limit)
Arias-Bujanda et al. (2018)	Spain	Cross-sectional Case–control	61 patients 13 patients	H_ns H_s	32 patients 41 patients	M-S-Ge-CP_ns M-S-Ge-CP_s	PPD, CAL, BOP, PI, BL Yes calibration	H: patients with BOP <25%, no sites with a PPD ≥4 mm and no radiographic evidence of alveolar BL	M-S-Ge-CP: patients with M to S Ge CP based on the previously established criteria (Armitage, 1999; Page & Eke, 2007)	Mean (total): 48.37 ± 11.55 y Mean (H): 45.65 ± 12.37 y Mean (M-S-Ge-CP): 51.12 ± 10.01 y	H: 61 non-smokers, 13 smokers M-S-Ge-CP: 32 non-smokers, 41 smokers	Pooled GCF samples were collected inserting a paper strip into the gingival sulcus or periodontal pocket for 30 seconds from 20 non-adjacent proximal sites per subject H: sites from teeth in quadrants 1 and 3 M-S-Ge-CP: sites from the deepest PPD sites in each quadrant (Periopaper; 300 mL of 0.01 M PBS; at −80°C)	IL1α (multiplex; 0.34–28,800 pg/mL) IL1β (multiplex; 0.09–23,150 pg/mL) IL2 (multiplex; 0.04–13,700 pg/mL) IL17A (multiplex; 0.36–30,900 pg/mL)
Baeza et al. (2016)	Chile	Cross-sectional Case–control	13 patients_31 sites	H	11 patients_31 sites	CP	PPD, CAL, BOP, BL, ALS Yes calibration	H: patients with PPD ≤3 mm in every site, BOP <10%, and absence of clinical diagnosis of asymptomatic apical P	CP: patients with ≥14 natural teeth excluding third molars, ≥5 sites with PPD ≥5 mm, CAL ≥3 mm and extensive radiographic BL	Mean (H): 44.1 ± 13.5 y Mean (CP): 50.8 ± 13.9 y	H: 15.4% smokers CP: 36.4% smokers	GCF samples were obtained a week after establishing the clinical diagnosis of CP by placing paper strips into de gingival sulcus or pocket for 30 seconds. H: samples obtained from each mesio-vestibular site from the first molars (total of 31 samples) CP: samples obtained from the deepest PPD sites (total of 31 samples) (Periopaper; 80 μL buffer/strip)	proMMP9 (gelatin zymography-densitometry) tMMP9 (gelatin zymography-densitometry)
Baliban et al. (2013)	United States of America	Cross-sectional Case–control	20 patients (from an initial group of 41)	H	21 patients (from an initial group of 55)	CP	PPD, CAL, BOP Yes calibration	H: patients considered H as previously described (Armitage, 1999; Baliban et al., 2012)	CP: patients considered to have CP as previously described (Armitage, 1999; Baliban et al., 2012)	Mean (H): 45.53 ± 6.97 y, range 34–46 Mean (CP): 48.02 ± 8.03 y, range 30–61	Non-smokers	Pooled GCF samples from 4 pre-selected sites. H: samples were taken from the mesio-buccal sites of first molars. CP: samples were taken from sites which displayed PPD >6 mm and <8 mm.	33 kDa chaperonin (LC–MS/MS) Angiotensinogen (LC–MS/MS) Carbonic anhydrase 1 (LC–MS/MS) Glyceraldehyde-3-phosphate dehydrogenase (LC–MS/MS)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of GCF biomarkers ($N = 10$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of GCF sample (storage)	Biomarkers evaluated (technique; detection limit)
												(100 μL of 100 mM ammonium bicarbonate; at −80°C)	Hb subunit delta (LC–MS/MS) Ig kappa chain V-I region AG (LC–MS/MS) L-lactate dehydrogenase A-like 6B (LC–MS/MS) Lysozyme C (LC–MS/MS) Peptidyl-prolyl cis-trans isomerase A (LC–MS/MS) Probable succinyl-CoA:3-ketoacid-coenzyme A transferase sub (LC–MS/MS) Prothrombin (LC–MS/MS) Ribulose bisphosphate carboxylase (LC–MS/MS) Thymidine phosphorylase (LC–MS/MS)
Beighton et al. (1990)	United Kingdom	Cross-sectional Case–control	21 patients_168 sites	H and G	22 patients_176 sites	CP	GI, PI, CAL, PPD, CI, BOP No calibration	H and G: patients with no CAL >3 mm at all designated sites, with BOP at no >2 of the designated sites	CP: patients with ≥4 of 8 designated sites (upper right and lower left second molar, lower right and upper left second premolar, upper right and lower left canine and lower right and upper left second incisor) with CAL >5 mm, together with BOP at those same sites	Mean (H and G): 32.2 ± 13.1 y Mean (CP): 37.0 ± 10.7 y	NS	GCF samples were collected from eight designated mesio-buccal tooth sites (upper right and lower left second molar, lower right and upper left second premolar, upper right and lower left canine, and lower right and upper left second incisor) introducing a 2 × 13 mm filter paper strip within the gingival crevice for 30 seconds. (Filter paper strip; 500 μL of ice-cold, 50 mM TES buffer; at ice)	Gly-proline-AMC (fluorogenic) L-arginine-AMC (fluorogenic) L-leucine-AMC (fluorogenic) N-alpha-benzoyl-L-arginine-AMC (fluorogenic) N-carbobenzoxy-gly-gly-arginine-AMC (fluorogenic) N-[p-toluenesulphonyl]-gly-pro-arginine-AMC (fluorogenic) N-succinyl-ala-phe-lysine-AMC (fluorogenic) N-tert-butoxycarbonyl-ileu-glut-gly-arginine-AMC (fluorogenic) N-tert-butoxycarbonyl-leu-ser-thr-arginine-AMC (fluorogenic) N-tert-butoxycarbonyl-val-leu-lysine-AMC (fluorogenic)

(Continues)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of GCF biomarkers ($N = 10$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of GCF sample (storage)	Biomarkers evaluated (technique; detection limit)
Beighton et al. (1992)	United Kingdom	Cross-sectional Case–control	20 patients_160 sites	H and G	20 patients_160 sites	CP	PI, GI, BOP, CI, CAL, PPD No calibration	H and G: patients with CAL ≤3 mm at any periodontal site (6 sites per tooth) and with BOP at no >2 of the designated sites	CP: patients with ≥4 of 8 designated mesio-buccal tooth sites (upper right and lower left 2nd molar, lower right and upper left second premolar, upper right and lower left canine and lower right and upper left second incisor) with CAL >5 mm, together with BOP at those same sites	Mean (H and G): 36.0 ± 15.3 y Mean (CP): 48.8 ± 10.6 y	NS	Individual GCF samples were collected from eight designated mesio-buccal tooth sites (upper right and lower left second molar, lower right and upper left second premolar, upper right and lower left canine, and lower right and upper left second incisor) by placing a 2 × 13 mm filter-paper strip just within the gingival crevice for 30 seconds (filter-paper strip; 500 μL of ice-cold, 50 mM TES buffer; at ice)	Alpha-glucosidase (fluorimeter) Alpha-L-fucosidase (fluorimeter) Alpha-mannosidase (fluorimeter) Beta-galactosidase (fluorimeter) Beta-glucosidase (fluorimeter) Beta-mannosidase (fluorimeter) Beta-N-acetylglucosaminidase (fluorimeter) Hexosaminidase A (fluorimeter) Sialidase (fluorimeter)
Hanioka et al. (2005)	Japan	Cross-sectional Cohort	18 patients_36 sites	SMD	9 patients_18 sites	SPD	PPD, CAL, PI, modified GI No calibration	SMD: Remaining subjects, including G subjects	SPD: patients with ≥1 site at which both PPD and CAL exceeded 3.5 mm	Mean (total): 41.8 ± 11.0 y	NS	GCF samples were collected with a paper strip at three fixed sites per subject (mesio-palatal of upper maxillary first molars and the deepest pocket within the mouth). A paper strip was inserted into the gingival crevice until resistance was encountered for 30 seconds. Analysis performed exclusively with data derived from maxillary first molars. (Periopaper; 1 mL of 0.05 M Tris–HCl buffer)	IgA (ELISA) Neutrophil elastase (ELISA)
Huang et al. (2020)	China	Cross-sectional Case–control	12 patients (from an initial group of 25)	H	12 patients (from an initial group of 25)	S-Ge-P	PPD, CAL, BOP No calibration	H: patients with good oral hygiene, clinically intact gingiva, and no clinical sign of gingival inflammation	S-Ge-P: patients with PPD ≥5 mm and CAL ≥4 mm	Mean (H): 38.36 ± 5.13 y, range 25–50 Mean (S-Ge-P): 40.93 ± 7.52 y, range 25–50	Non-smokers	GCF sample was collected using a 2 × 10 mm filter paper strip that was inserted into the gingival crevice for 30 seconds. S-Ge-P: sample was taken from five pre-selected	IL1β (antibody array; 3.9 pg/mL) IL8 (antibody array; 1.8 pg/mL) MMP13 (antibody array; 43.7 pg/mL) OA (antibody array; 74.1 pg/mL)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of GCF biomarkers ($N = 10$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of GCF sample (storage)	Biomarkers evaluated (technique; detection limit)
												sites which displayed PPD between 6 and 8 mm. (Filter paper strip; 100 μL of PBS; at −80°C)	OPG (antibody array; 15.7 pg/mL)
Kitamura et al. (1991)	Japan	Longitudinal Case–control	10 patients_13 sites	CP_Non-CAL	10 patients_13 sites	CP_CAL	CAL, PPD, GI, BOP, PI No calibration	CP: patients with untreated CP Non-CAL: inactive sites with comparable PPD to the active sites, but CAL did not change during period of 42 d (CAL <2 mm)	CP: patients with untreated CP CAL: active sites with CAL ≥2 mm between 0 and 7 d, and between 35 and 42 d	NS	NS	Individual GCF samples were collected from active and inactive sites on 42 d (GCF diluted on pyrogen-free distilled water)	aCOL (NS) lCOL (NS) tCOL (NS) IL1α (NS) IL1β (NS) LPS (NS) PGE2 (NS)
Nakashima et al. (1996)	Switzerland	Interventional Cohort	8 patients_43 sites	CP_Non-CAL	8 patients_10 sites	CP_CAL	CAL, PPD, GI, BOP at baseline, 3, 6, 9 and 12 mo. PPD and CAL during the first and second week, GI and BOP only the first week. No calibration	CP: patients with ≥4 premolars and/or molars with CAL >5 mm Non-CAL: inactive sites with negligible CAL during the same period (belonging to the same oral cavity)	CP: patients with ≥4 premolars and/or molars with CAL >5 mm CAL: active sites with CAL ≥1.5 mm per 3 mo, and reconfirmed after 3 further mo	Mean (CP): 43.1 y	NS	Individual GCF samples were collected at baseline, 3, 6, 9 and 12 mo from 4 sites (mesio- and disto-buccal, mesio- and disto-lingual) of premolars and molars. PMNs count (collected 1st week of each time point): (1 durapore strip/sulcus for 10 seconds; 40 mL of PBS; immediately used for PMNs count) Biochemical analysis (collected second week of each time point): (2 durapore strips/sulcus for 1 min; 250 μL of physiological saline; at −70°C)	a2M (ELISA) AEL (ELISA) ALP (Spectrophotometer) COL (modified Lamster method) OC (ELISA) PGE2 (ELISA) PMN (Neubauer Chamber)
Tomás et al. (2017)	Spain	Cross-sectional Case–control	74 patients	H	73 patients	M-S-Ge-CP	PPD, CAL, BOP, PI, BL Yes calibration	H: patients with BOP <25%, no sites with a PPD ≥4 mm and no radiographic evidence of alveolar BL	M-S-Ge-CP: patients with M to S Ge CP based on the previously established criteria (Armitage, 1999; Page & Eke, 2007)	Mean (total): 48.37 ± 11.55 y Mean (H): 45.65 ± 12.37 y Mean (M-S-Ge-CP): 51.12 ± 10.01 y	H: 61 non-smokers, 13 smokers M-S-Ge-CP: 32 non-smokers, 41 smokers	Pooled GCF samples were collected from 20 sites per subject H: sites from teeth in quadrants 1 and 3 (incisor, canine, first premolar, second premolar, and molar) M-S-Ge-CP: sites from the deepest PPD sites in each quadrant (a total of 20 non-	IFNγ (multiplex; 0.02–6650 pg/mL) IL1α (multiplex; 0.34–28,800 pg/mL) IL1β (multiplex; 0.09–23,150 pg/mL) IL10 (multiplex; 0.04–10,050 pg/mL) IL17A (Multiplex; 0.36–30,900 pg/mL)

(Continues)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of GCF biomarkers (*N* = 10 articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of GCF sample (storage)	Biomarkers evaluated (technique; detection limit)
												adjacent proximal sites) (Periopaper; 300 mL of 0.01 M of PBS; at −80°C)	

Main characteristics of the articles that analysed combinations of salivary biomarkers (*N* = 13 articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of saliva sample (storage)	Biomarkers evaluated (technique; detection limit)
Altıngöz et al. (2021)	Turkey	Cross-sectional Case–control	28 patients	H	26 patients	CP	PI, GI, PPD, CAL, BOP Yes calibration	H: patients with PPD ≤3 mm, CAL ≤1 mm, no gingival inflammation, good oral hygiene	CP: patients with ≥16 teeth, ≥8 sites with PPD ≥6 mm, <4 sites with CAL ≥5 mm in ≥2 quadrants. Patients with P III (Tonetti et al., 2018)	Mean (H): 44.8 ± 11.5 y Mean (CP): 46.1 ± 5.3 y	H: 23 non-smokers, 5 smokers CP: 20 non-smokers, 6 smokers	Unstimulated whole saliva (at −80°C)	4-HNE (ELISA) 8-OHdG (ELISA) hsCRP (ELISA) MDA (ELISA)
Ebersole et al. (2013)	United States of America	Longitudinal Cohort	30 patients	H	50 patients	CP	PPD, BOP, CAL Yes calibration	H: patients with ≥20 teeth, BOP <10%, PPD ≥5 mm in <2% of sites, no PPD ≥6 mm, CAL >2 mm in <1% of sites	CP: patients with 5 qualifying sites in 2 quadrants with ≥2 affected teeth in each quadrant with each site having PPD ≥5 mm, CAL ≥3 mm, BOP score ≥2	Mean (H): 31.4 ± 6.8 y, range: 18–45 y Mean (CP): 43.0 ± 10.8 y	H: non-smokers CP: 28% smokers	Unstimulated whole saliva (at −80°C)	IL1β (multiplex) IL6 (multiplex) MMP8 (ELISA)
Ebersole et al. (2015)	United States of America	Cross-sectional Case–control	65 patients 43 patients	H G	101 patients	P	PPD, BOP, CAL No calibration	H: patients with BOP ≤10% of sites (6 sites/tooth), <3% of sites with PPD ≥4 mm, no CAL ≥2 mm G: patients with BOP ≥20%, <3% of sites with PPD ≥4 mm, no CAL ≥2 mm	P: patients with BOP >10%, with >5% of sites with PPD ≥4 mm and CAL ≥2 mm	Mean (H): 28.2 ± 5.9 y Mean (G): 27.8 ± 4.5 y Mean (P): 42.0 ± 10.4 y	H: non-smokers G: non-smokers P: 28 smokers	Unstimulated whole saliva (at −80°C)	IL1β (multiplex) IL6 (multiplex; 0.64 pg/mL) MIP1α (multiplex) MMP8 (multiplex)
Ferrando et al. (2005)	Spain Sweden	Cross-sectional Case–control	18 patients	H	27 patients	CP	CPITN No calibration	H: CPITN was used to evaluate the periodontal state	CP: CPITN was used to evaluate the periodontal state	Mean (G): 29 ± 10 y Mean (CP): 44 ± 11 y	NS	Saliva (at −30°C)	3-OH-C12:0 (GC–MS/MS) 3-OH-C14:0 (GC–MS/MS) 3-OH-Ci17:0 (GC–MS/MS) 3-OH-C17:0 (GC–MS/MS)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of salivary biomarkers ($N = 13$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of saliva sample (storage)	Biomarkers evaluated (technique; detection limit)
Görgülü and Doğan (2022)	Turkey	Cross-sectional Case–control	20 patients	H	20 patients 25 patients	P_III-B P_III-C	PI, GI, BOP, PPD, CAL, BL Yes calibration	H: patients H (Tonetti et al., 2018). BOP <10%, PPD ≤3 mm, no sites with interdental CAL, no BL, no history of P	P_III-B, P_III-C: patients with P III (Tonetti et al., 2018). ≥4 interdental sites with CAL ≥5 mm, PPD ≥6 mm, BOP ≥30%, BL ≥1/3 root, ≥4 tooth loss due to P P_III-B: % BL/Age 0.25–1.0 P_III-C: % BL/Age >1.0	Median (H): 32.5 y Q1–Q3 (H): 29.25–43.25 y Median (P_III-B): 39.00 y Q1–Q3 (P_III-B): 34.75–43.75 y Median (P_III-C): 33.00 y Q1-Q3 (P_III-C): 30.00–37.50 y	Non-smokers	Unstimulated whole saliva (at −80°C)	IL34 (ELISA; 15.6 pg/mL) MAF (ELISA; 0.2 ng/mL) MIP1α (ELISA; 23.44 pg/mL) MMP8 (ELISA; 0.16 ng/mL)
Grant et al. (2022)	United Kingdom	Cross-sectional Case control	74 patients	H_Group1, H_Group2, G_Group1 and G_Group2	80 patients	P_I-II_Group1, P_I-II_Group2, P_III-IV_Group1 and P_III-IV_Group2	CAL, PPD, GI, BOP No calibration	H_Group1: patients with no CAL, no PPD >3 mm, <10% GI of 1 and no GI of 2–3, BOP <10% H_Group2: patients with no CAL, PPD ≤3 mm (≤4 mm at distal of last molars), ≤10% GI ≥2, BOP <10% G_Group1: patients with no CAL, >30% GI >2, BOP 30%, no PPD >4 mm G_Group2: patients with no CAL, >30% GI ≥3, BOP >10%, no PPD >4 mm	P_I-II_Group1: patients with interproximal CAL 2-4 mm at >8 teeth with PPD 5-7 mm P_I-II_Group2: patients with interproximal PPD 5–7 mm (≈CAL 2-4 mm) at ≥8 teeth, BOP >30% P_III-IV_Group1: patients with interproximal CAL 5 mm at >12 teeth and PPD >7 mm P_III-IV_Group2: patients with interproximal PPD >7 mm (≈CAL ≥5 mm) at ≥12 teeth, BOP >30%	Mean (H_Group1): 39 ± 9 y Mean (H_Group2): 35 ± 11.9 y Mean (G_Group1): 38 ± 11 y Mean (G_Group2): 32.8 ± 9.7 y Mean (P_I-II_Group1): 47 ± 6 y Mean (P_I-II_Group2): 43.8 ± 7.2 y Mean (P_III-IV_Group1): 49 ± 7 y Mean (P_III-IV_Group2): 43.8 ± 7.2 y	Non-smokers	Stimulated whole saliva with sterilised marble (at −80°C)	A1AGP (ELISA) MMP9 (ELISA) PK (ELISA) S100A8 (ELISA)
Inönü et al. (2020)	Turkey	Cross-sectional Case control	90 patients	H and G	90 patients	CP and AgP	PI, GI, PPD, CAL, BOP, BL No calibration	H: patients with PPD ≤3 mm, BOP ≤10% of sites (6 sites/tooth), no gingival redness or edema, no alveolar crestal BL G: patients with GI >0, PPD ≤3 mm at ≥90% of teeth, no alveolar BL, no CAL	CP: patients with CAL ≥4 mm, PPD ≥5 mm at ≥4 teeth in each jaw and ≥50% alveolar BL in ≥2 quadrants AgP: patients with AgP as previously described (Armitage, 1999)	Mean (total): 32.41 ± 9.38 y, range: 20–78 y Mean (H): 28.0 y, range: 25.0–41.0 y Mean (G): 24.5 y, range: 20.0–56.0 y Mean (CP): 43.5 y, range: 27.0–58.0 y Mean (AgP): 28.0 y, range: 20.0–35.0 y	Non-smokers	Unstimulated whole saliva (at −80°C)	Del-1 (ELISA) IL17 (ELISA) LFA-1 (ELISA)

(Continues)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of salivary biomarkers ($N = 13$ articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of saliva sample (storage)	Biomarkers evaluated (technique; detection limit)
Kim et al. (2021)	Korea	Cross-sectional Case–control	92 patients	H	129 patients	P	PI, PPD, CAL, BOP, GI No calibration	H: patients without detectable inter-dental CAL	P: patients with P II-III (Tonetti et al., 2018)	Mean (H): 29.93 ± 7.85 y Mean (P): 58.34 ± 11.12 y (stage II), 56.00 ± 8.74 y (stage III)	Non-smokers	Stimulated saliva with plain cotton roll (at −80°C)	Butyrate (H-NMR spectroscopy) Ethanol (H-NMR spectroscopy) Glucose (H-NMR spectroscopy) Isovalerate (H-NMR spectroscopy) Taurine (H-NMR spectroscopy)
Nagarajan et al. (2015)	United States of America	Cross-sectional Case–control	40 patients	G	40 patients	P	BOP, PPD, CAL No calibration	G: patients with BOP at ≥20% of sites (6 sites/tooth), <10% of sites with PPD ≥4 mm and no sites with CAL ≥2 mm	P: patients with BOP at >20% of sites, >10% of sites with PPD ≥4 mm and CAL ≥2 mm	Mean (G): 27.5 ± 4.5 y Mean (P): 40.8 ± 10.5 y	G: non-smokers P: 27% smokers	Unstimulated whole saliva (at −80°C)	IL1β (multiplex) IL6 (multiplex) MIP1α (multiplex) MMP8 (multiplex)
Ochanji et al. (2017)	Kenya	Cross-sectional Case–control	77 patients	G	81 patients	Mi-M-S-P	BOP, PI, PPD, REC, CAL, GI Yes calibration	G: patients with no periodontal disease based on the consensus CDC/AAP.	Mi-M-S-P: patients with periodontal disease based on the consensus CDC/AAP.	Mean (total): 37 ± 12.74 y, range: 18–75 y	136 non-smokers 22 smokers	Unstimulated whole saliva (−70°C)	OPG (ELISA) RANKL (ELISA)
Rzeznik et al. (2017)	France	Cross-sectional Case–control	25 patients	H	26 patients	CP and AgP	PPD, CAL, BL, BOP, DMF, NRT, PI, GI Yes calibration	H: patients with irrelevant clinical parameters as they were healthy subjects free of any P	CP and AgP: patients with ≥2 interproximal sites with CAL ≥3 mm and ≥2 interproximal sites with PPD ≥4 mm (not on the same tooth) or one site with PPD ≥5 mm. Localised if ≤30% and generalised if >30% sites affected	Mean (H): 40.7 ± 12.4 y Mean (CP and AgP): 42.4 ± 12.8 y	H: 12 non-smokers, 2 former, 11 smokers CP and AgP: 13 non-smokers, 2 former, 11 smokers	Stimulated saliva with paraffin (at −25°C)	Butyrate (H-NMR spectroscopy) GABA (H-NMR spectroscopy) Lactate (H-NMR spectroscopy)
Wu et al. (2018)	Taiwan	Cross-sectional Case–control	27 patients	No-P	30 patients	P	PPD, CAL, REC, BL No calibration	No-P: patients with no teeth exhibiting CAL >3 mm and with no radiographic BL.	P: patients who fulfilled the requirement of generalised CP in accordance with the classification defined by the AAP in 1999.	Mean (No-P): 40.63 ± 16.50 y Median (No-P): 34.67 y Mean (P): 44.12 ± 12.00 y Median (P): 43.25 y	Non-smokers	Unstimulated whole saliva (at −20°C)	IL1β (multiplex) IL1ra (multiplex) MMP8 (ELISA) MMP9 (ELISA) TNFα (multiplex)

TABLE 1 (Continued)

Main characteristics of the articles that analysed combinations of salivary biomarkers (*N* = 13 articles)

Author (year)	Country	Type of study	No. of control condition	Type of control condition	No. of target condition	Type of target condition	Standard reference	Diagnostic criteria for control condition	Diagnostic criteria for target condition	Age in total or by groups	Smoking total or by groups	Type of saliva sample (storage)	Biomarkers evaluated (technique; detection limit)
Zhang et al. (2021)	China	Cross-sectional Case–control	25 patients 24 patients	H G	31 patients	P	PI, BOP, PPD, CAL, BL No calibration	H: patients with BOP <10%, PPD ≤3 mm, no CAL, no BL, no signs of inflammatory lesions in oral mucosa G: patients with BOP ≥10%, PPD ≤3 mm, no CAL, no BL	P: patients with interdental CAL ≥5 mm, PPD ≥6 mm, BL ≥2/3 of the root, loss of ≤4 teeth due to P. Patients with P III-C (Tonetti et al., 2018)	Mean (H): 24.68 ± 3.52 y Mean (G): 26.32 ± 4.02 y Mean (P): 42.58 ± 3.39 y	Non-smokers	Unstimulated whole saliva (at −80°C)	ICTP (ELISA) IL1β (ELISA) MMP8 (ELISA) Pg (qPCR)

Abbreviations in alphabetical order, excluding the name of the biomarkers: µL, microlitre; AAP, American Academy of Periodontology; Ag, aggresive; ALS, apical lesion size; BL, bone loss; BOP, bleeding on probing; CAL, clinical attachment loss; CDC, centers for disease control; CI, calculus index; CP, chronic periodontitis; CPITN, community periodontal index of treatment needs; d, day(s); DMF, decay missing filled; ELISA, enzyme-linked immunosorbent assay; G, gingivitis; GC, gas chromatography; GCF, gingival crevicular fluid; Ge, generalised; GI, gingival index; H, periodontally healthy; H-NMR, nuclear magnetic resonance; LC, liquid chromatography; M, moderate; Mi, mild; min, minute(s); mL, millilitre; mm, millimetre; mo, month(s); MS/MS, tandem mass spectrometry; Multiplex, multiparametric cytometry; ng, nanograms; No., number; NRT, number of residual teeth; NS, not specified; ns, non-smokers; P, periodontitis; PBS, phosphate-buffered saline; pg, picogram; PI, plaque index; PPD, probing pocket depth; qPCR, quantitative polymerase chain reaction; REC, gingival recession; S, severe; s, smokers; SMD, subject with minimal periodontal destruction; SPD, subject with periodontal destruction; TES, N-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid; Tris–HCl, tris(hydroxymethyl)aminomethane hydrochloride; y, year(s).

Abbreviations of the names of the biomarkers in alphabetical order: 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; a, active; A1AGP, alpha-1-acid glycoprotein; a2M, alpha-2 macroglobulin; AEL, antigenic elastase; ALP, alkaline phosphatase; COL, collagenase; Del, developmental endothelial locus; GABA, gamma aminobutyric acid; Hb, haemoglobin; hsCRP, high sensitivity C-reactive protein; ICTP, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; IFN, interferon; Ig, immunoglobulin; IL, interleukin; l, latent; LFA, lymphocyte function-associated antigen; LPS, lipopolysaccharide; MAF, macrophage-activating factor; MDA, malondialdehyde; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; OA, osteoactivin; OC, osteocalcin; OPG, osteoprotegerin; Pg, *Porphyromonas gingivalis*; PGE2, prostaglandin E2; PK, pyruvate kinase; PMN, polymorphonuclear leukocytes; pro, proform; ra, receptor antagonist; RANKL, receptor activator nuclear kappa ligand; t, total; TNF, tumour necrosis factor.

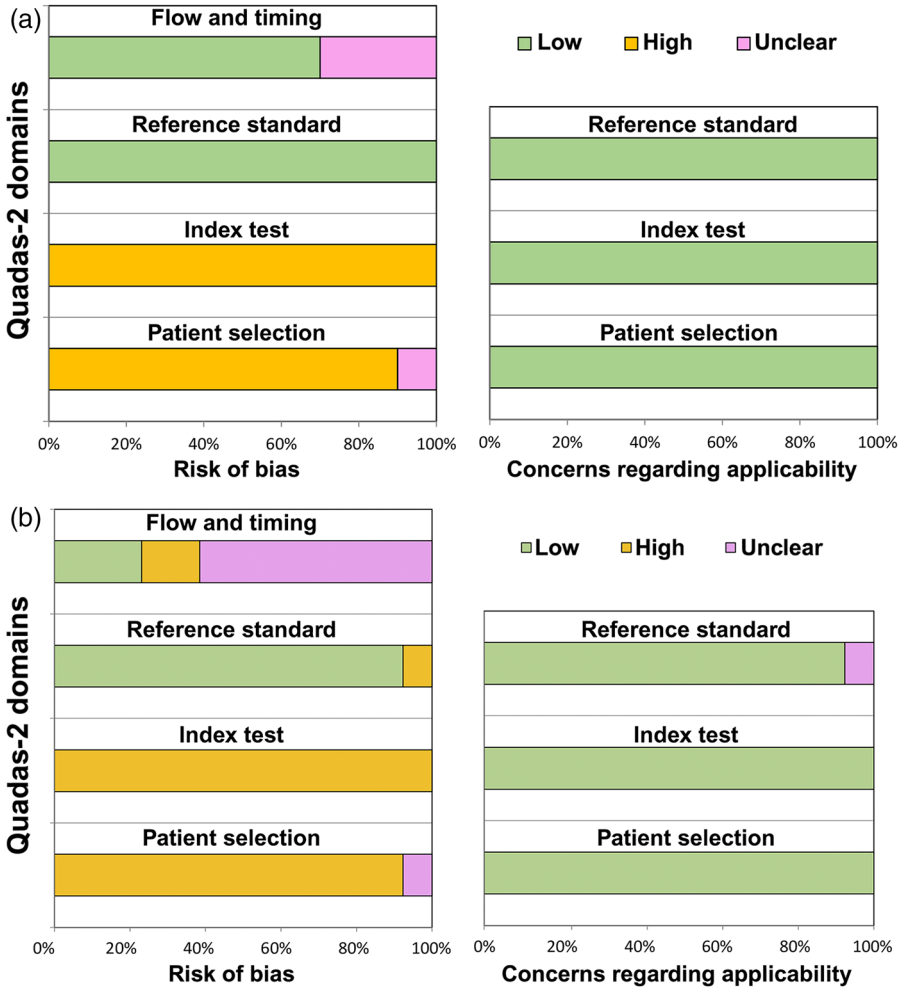

FIGURE 1 Quality assessment of the diagnostic accuracy studies included in (a) gingival crevicular fluid and (b) saliva according to the modified QUADAS-2 tool.

3.4 | Synthesis of qualitative analyses of GCF biomarker combinations

In GCF it was not feasible to perform meta-analysis according to the pre-defined criteria, as the associations of the 21 biomarkers were assessed in a single contingency table.

Considering those combinations that showed ACC values >95% (sensitivity/specificity values), Baliban et al. (2013) obtained the highest levels with an ACC of 96.4% (95.2%/97.6%) for four different combinations of 10 biomarkers each, followed by Huang et al. (2020) with an ACC of 96% (96.0%/96.0%) for the association of five biomarkers (interleukin IL-1β, IL-8, matrix metalloproteinase MMP-13, osteoactivin [OA] and osteoprotegerin [OPG]).

Regarding the combinations of two molecules that gave an ACC >90%, the association of two cytokines (IL-1α, IL-1β or IL-17A with interferon IFN-γ or IL-10) showed ACC values of 92.5%–95.2% (90.4%–94.5%/94.6%–97.3%) (Tomás et al., 2017). In non-smokers, IL-1α, IL-1β or IL-17A with cytokine IL-2 as a ratio gave ACC values of 80.6%–88.2% (81.3%–87.5%/77.0%–90.2%), although in smokers it increased to 84.4%–92.6% at the expense of sensitivity (95.1%–98.8%). However, the specificity values were lower (38.5%–84.6%) (Arias-Bujanda et al., 2018) (Figure 2, Appendix S3.2).

3.5 | Synthesis of qualitative and quantitative analyses of six salivary biomarker combinations

The ACC ranges (sensitivity/specificity ranges) for all salivary combinations are detailed in Appendices S3.3 and S3.4. Meta-analysis was performed in 6 of the 47 salivary biomarker combinations because they were the only ones that presented three or more contingency tables in two or more different articles (Figures 3 and 4; Appendices S8 and S9).

The association of IL-1β with MMP-8 was the most studied (Ebersole et al., 2013, 2015; Zhang et al., 2021), followed by IL-1β with IL-6; IL-6 with MMP-8 (Ebersole et al., 2013, 2015); macrophage inflammatory protein (MIP)-1α with MMP-8 (Ebersole et al., 2013; Görgülü & Doğan, 2022), IL-1β, IL-6 and MMP-8 (Ebersole et al., 2013, 2015) and the combination of these four biomarkers (IL-1β, IL-6, MIP-1α and MMP-8) (Ebersole et al., 2013; Nagarajan et al., 2015). Regarding the type of biomarkers, these molecules were pro-inflammatory and host-response mediators (IL-1β, IL-6 and MIP-1α) and an enzyme (MMP-8).

Regarding the HSROC modelling, the combinations ordered from highest to lowest sensitivity estimations ± standard deviation were as follows: IL-6 with MMP-8 (86.2 ± 23.1%); IL-1β with IL-6 (83.0 ± 21.0%); IL-1β with MMP-8 (82.7 ± 16.1%); IL-1β, IL-6 and MMP-8 (81.8 ± 21.5%); IL-1β, IL-6, MMP-8 and MIP-1α (76.6 ± 15.0%);

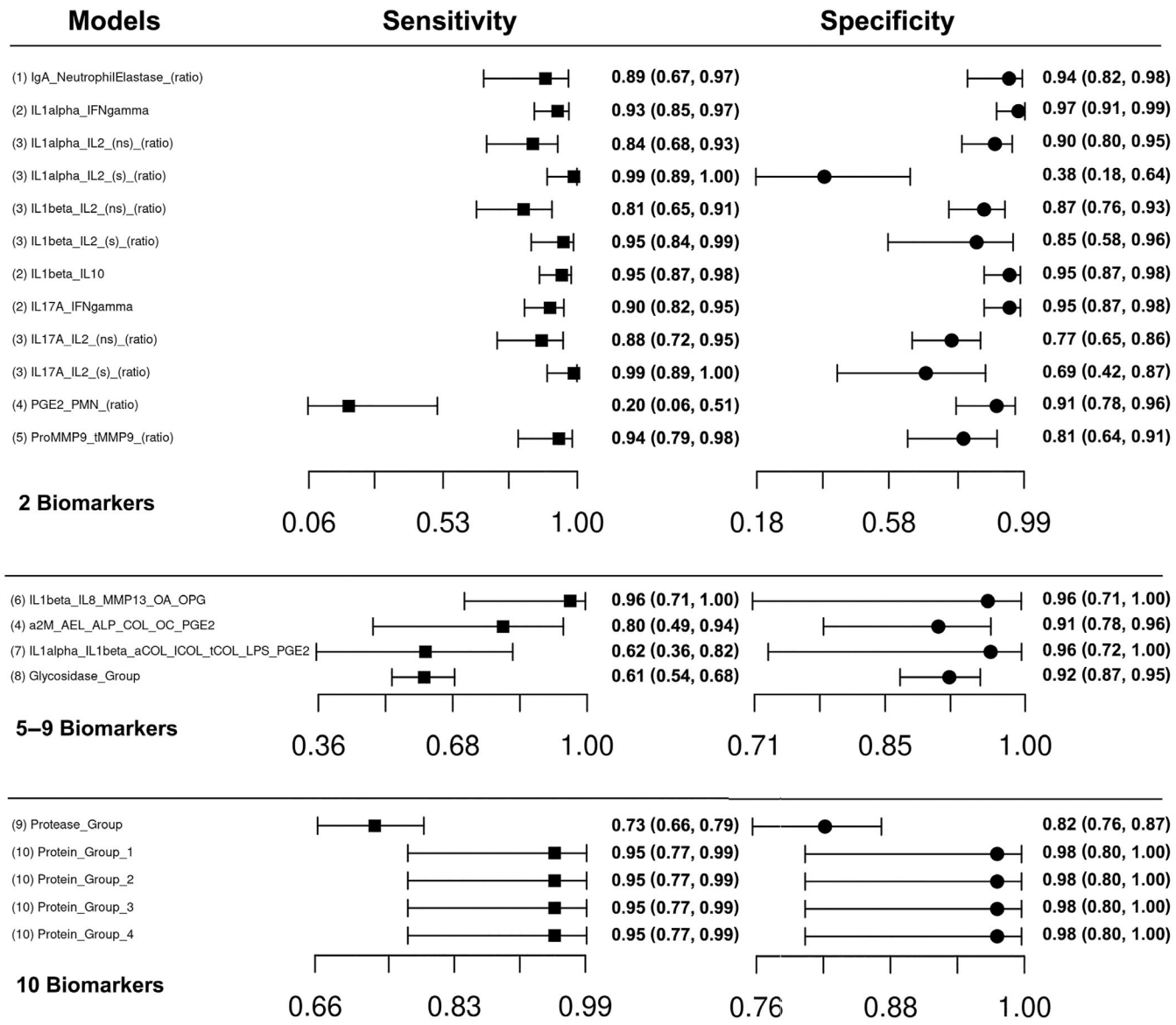

FIGURE 2 Forest plot with diagnostic test accuracy results (sensitivity, specificity and 95% confidence intervals) of each combination of gingival crevicular fluid biomarkers included. (1) Hanioka et al. (2005); (2) Tomás et al. (2017); (3) Arias-Bujanda et al. (2018); (4) Nakashima et al. (1996); (5) Baeza et al. (2016); (6) Huang et al. (2020); (7) Kitamura et al. (1991); (8) Beighton et al. (1992); (9) Beighton et al. (1990) and (10) Baliban et al. (2013). In the forest plot, sensitivity and specificity values are rounded, and the combinations of biomarkers are listed for a number of biomarkers (from lowest to highest) and in alphabetical order. If the data of true positive, true negative, false positive and false negative values for each contingency table of a biomarker were not detailed in the article, the authors calculated this table by considering the sensitivity and specificity values and the sample size of the control and target groups. In relation to the precise sensitivity and specificity values provided in the selected articles, the values of the calculated contingency tables mostly showed decimal values, which had to be rounded and sensitivity and specificity values recalculated. Values equal to zero were not allowed in the tables and were replaced by 0.5; in this way, we avoided infinite values in some measures of the binary classification test, for example, in the diagnostic odds ratio or the positive likelihood value. a, active; a2M, alpha-2 macroglobulin; AEL, antigenic elastase; ALP, alkaline phosphatase; COL, collagenase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; l, latent; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; ns, non-smokers; OA, osteoactivin; OC, osteocalcin; OPG, osteoprotegerin; PGE2, prostaglandin E2; PMN, polymorphonuclear leukocytes; pro, proform; s, smokers; t, total.

and MIP-1α with MMP-8 (71.0 ± 21.3%). Considering the specificity estimations, the values were (from highest to lowest) as follows: IL-1β, IL-6 and MMP-8 (84.3 ± 20.8%); IL-1β with IL-6 (83.7 ± 20.5%); IL-1β with MMP-8 (80.8 ± 16.7%); IL-6 with MMP-8 (80.5 ± 23.7%); IL-1β, IL-6, MMP-8 and MIP-1α (79.7 ± 14.7%); and MIP-1α with MMP-8 (75.6 ± 20.9%) (Figure 5, Appendix S9).

Considering these results, the clinical efficacy of these six combinations and a 45% prevalence of periodontitis (Eke et al., 2020; Tonetti et al., 2015), 80.7% of the IL-1β with IL-6 positive tests would indicate a true positive, and 85.8% of the negative tests would imply a true negative. These amount to 78.4% and 87.7% for IL-6 with MMP-8; 77.9% and 85.1% for IL-1β with MMP-8; and 70.4% and 76.1% for

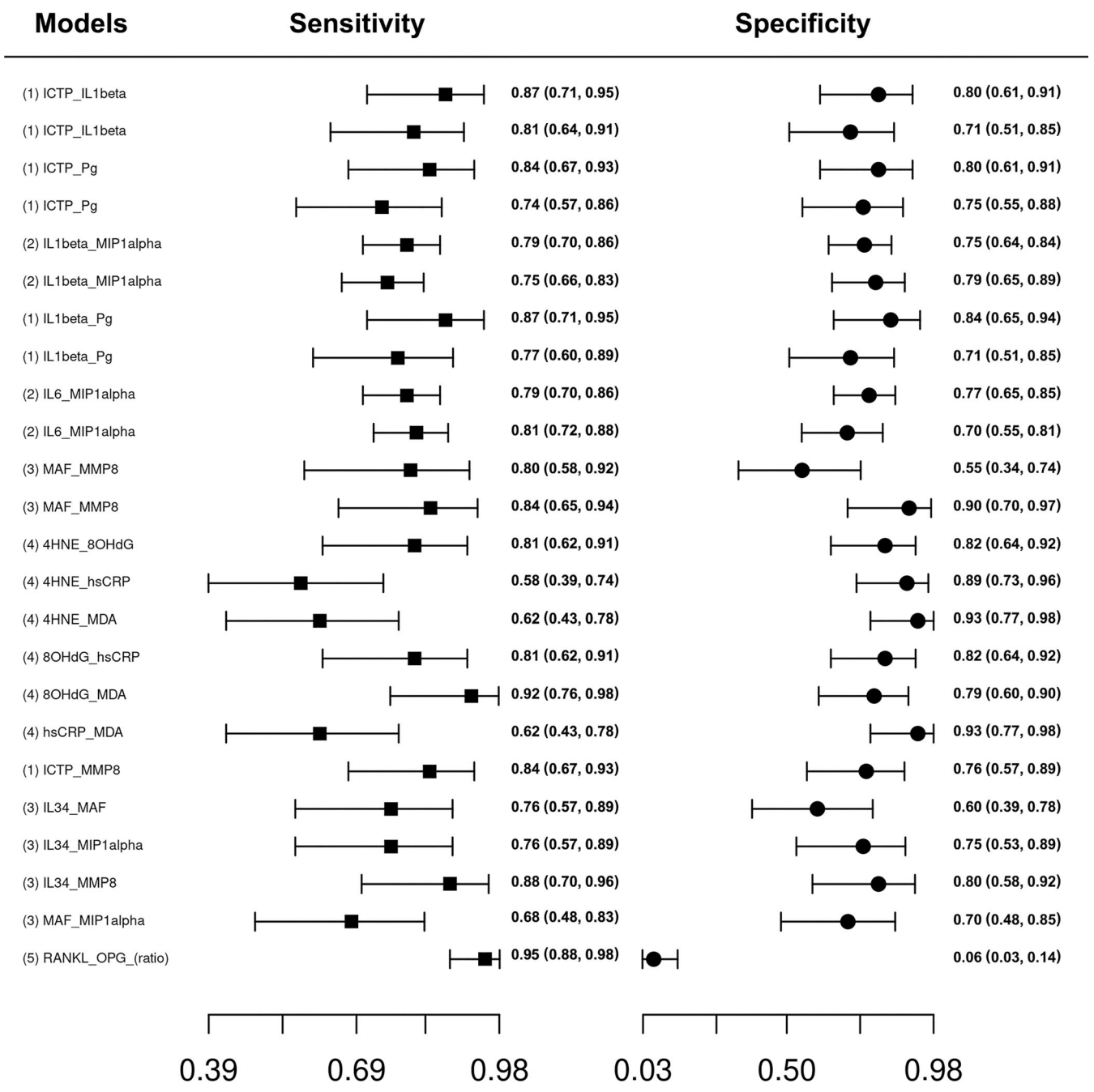

FIGURE 3 Forest plot with diagnostic test accuracy results (sensitivity, specificity and 95% confidence intervals) of each combination of two salivary biomarkers evaluated in less than three contingency tables. (1) Zhang et al. (2021); (2) Ebersole et al. (2015); (3) Görgülü and Doğan (2022); (4) Altıngöz et al. (2021) and (5) Ochanji et al. (2017). In the forest plot, sensitivity and specificity values are rounded, and the combinations of biomarkers are listed for a number of contingency tables and in alphabetical order. If the data of true positive, true negative, false positive and false negative values for each contingency table of a biomarker were not detailed in the article, the authors calculated this table considering the sensitivity and specificity values and the sample size of the control and target groups. In relation to the precise sensitivity and specificity values provided in the selected articles, the values of the calculated contingency tables mostly showed decimal values, which had to be rounded and sensitivity and specificity values recalculated. Values equal to zero were not allowed in the tables and were replaced by 0.5; in this way, we avoided infinite values in some measures of the binary classification test, for example, in the diagnostic odds ratio or the positive likelihood value. 4HNE, 4-hydroxy-2-nonenal; 8OHdG, 8-hydroxy-2′-deoxyguanosine; hsCRP, high sensitivity C-reactive protein; ICTP, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; IL, interleukin; MAF, macrophage-activating factor; MDA, malondialdehyde; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; OPG, osteoprotegerin; Pg, *Porphyromonas gingivalis*; RANKL, receptor activator nuclear kappa ligand.

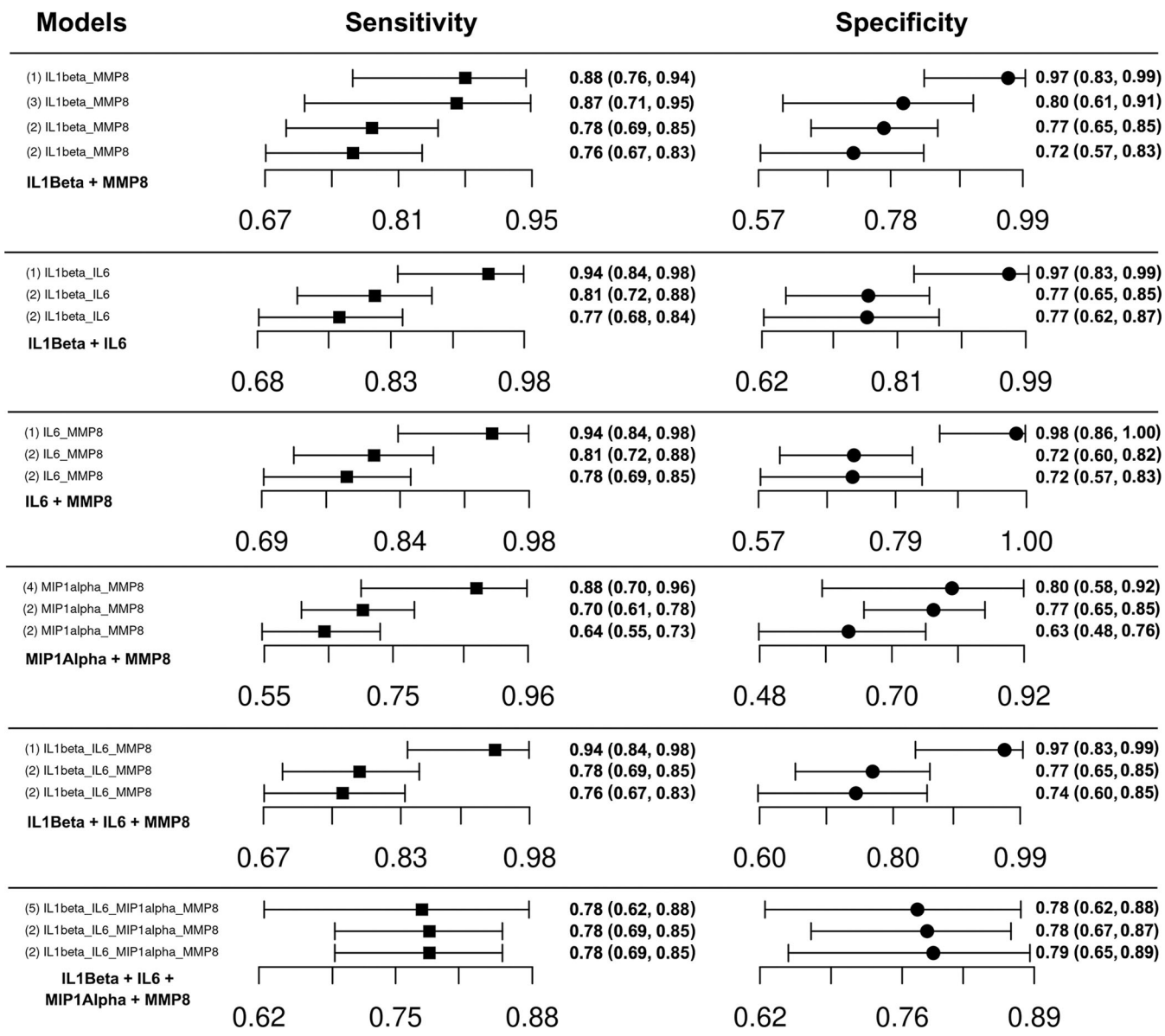

FIGURE 4 Forest plot with diagnostic test accuracy results (sensitivity, specificity and 95% confidence intervals) of each combination of salivary biomarkers evaluated in at least three contingency tables in at least two studies. (1) Ebersole et al. (2013); (2) Ebersole et al. (2015); (3) Zhang et al. (2021); (4) Görgülü and Doğan (2022) and (5) Nagarajan et al. (2015). In the forest plot, sensitivity and specificity values are rounded, and the combinations of biomarkers are listed according to the sensitivity value (from highest to lowest value). If the data of true positive, true negative, false positive and false negative values for each contingency table of a biomarker were not detailed in the article, the authors calculated this table considering the sensitivity and specificity values and the sample size of the control and target groups. In relation to the precise sensitivity and specificity values provided in the selected articles, the values of the calculated contingency tables mostly showed decimal values, which had to be rounded and sensitivity and specificity values recalculated. Values equal to zero were not allowed in the tables and were replaced by 0.5; in this way, we avoided infinite values in some measures of the binary classification test, for example, in the diagnostic odds ratio or the positive likelihood value. IL, interleukin; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase.

MIP-1α with MMP-8. For the combination of IL-1β, IL-6 and MMP-8, it would be 81.0% and 85.0%, whereas for the association of the four biomarkers, it would be 75.5% and 80.6% (Figure 6).

4 | DISCUSSION

This systematic review/meta-analysis evaluated the diagnostic accuracy of 21 biomarker combinations in GCF and 47 in saliva. Although it was not possible to perform a meta-analysis with GCF biomarkers, there were combinations of two cytokines (IL-1α, IL-1β or IL-17A with IFN-γ or IL-10) that showed sensitivity and specificity of >90%. The six salivary combinations evaluated meta-analytically were (mean sensitivity/specificity) the following: IL-6 with MMP-8 (86.2%/80.5%), IL-1β with IL-6 (83.0%/83.7%), IL-1β with MMP-8 (82.7%/80.8%), MIP-1α with MMP-8 (71.0%/75.6%), IL-1β, IL-6 and MMP-8 (81.8%/84.3%), and IL-1β, IL-6, MIP-1α and MMP-8 (76.6%/79.7%).

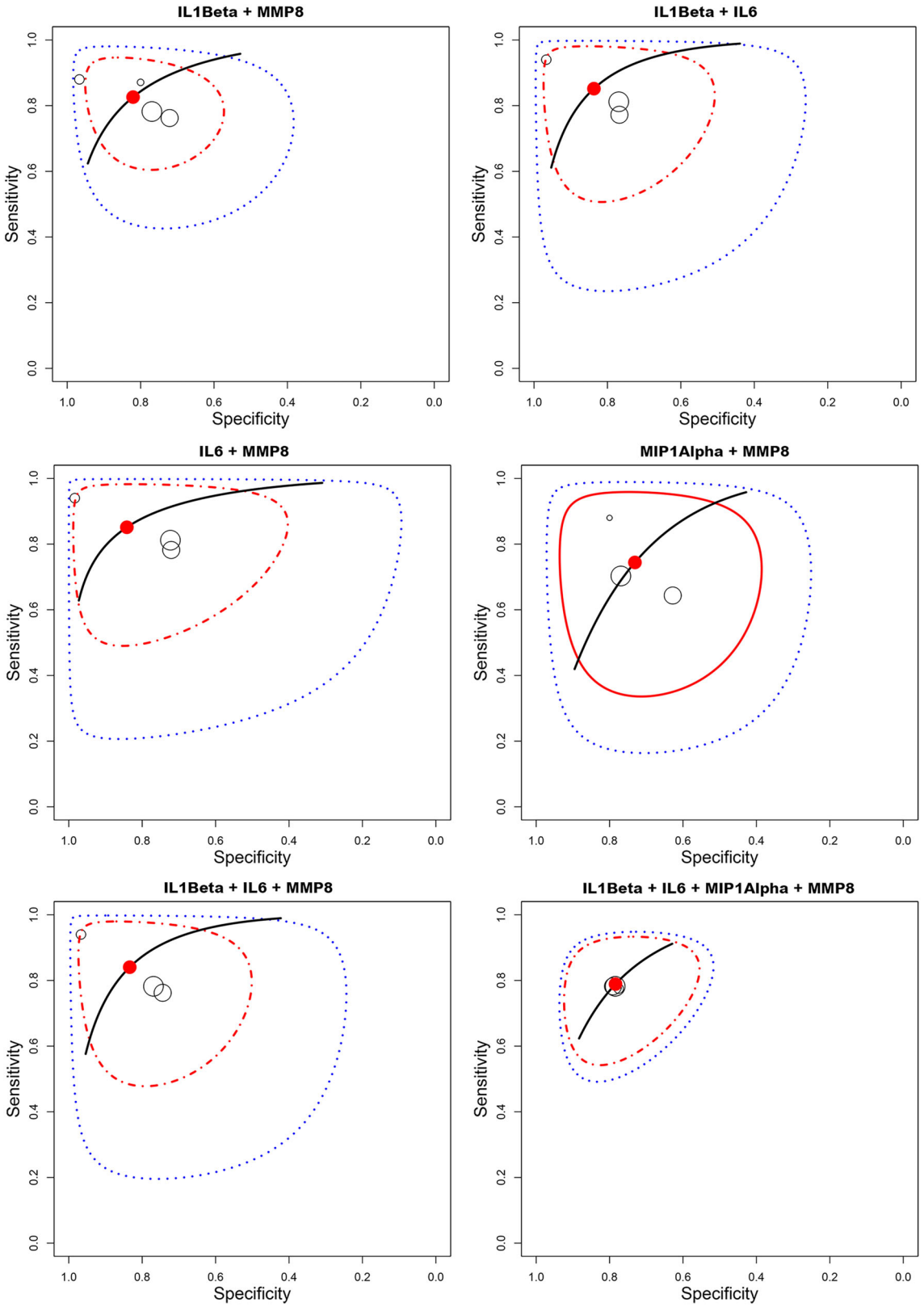

FIGURE 5 Meta-analyses performed on the six combinations of salivary biomarkers using hierarchical summary receiver operating characteristic (HSROC) modelling. HSROC includes summary points of sensitivity and specificity (median values; red-filled point), together with their prediction and confidence region. The prediction region (red dotted area) refers to potential sensitivity and specificity values that might be found in a future study by describing the full extent of the uncertainty of the summary points. This region can represent between-study heterogeneity (Harbord et al., 2007). The confidence region (blue red dotted area) is associated with the summary estimates of sensitivity and specificity jointly in the HSROC space, while it also accounts for their inverse association based on the included studies. This region does not, however, reflect the between-study heterogeneity (Dinnes et al., 2005). Each black circle represents a classification of a biomarker included in the meta-analysis, and its size is proportional to the sample size. IL, interleukin; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase.

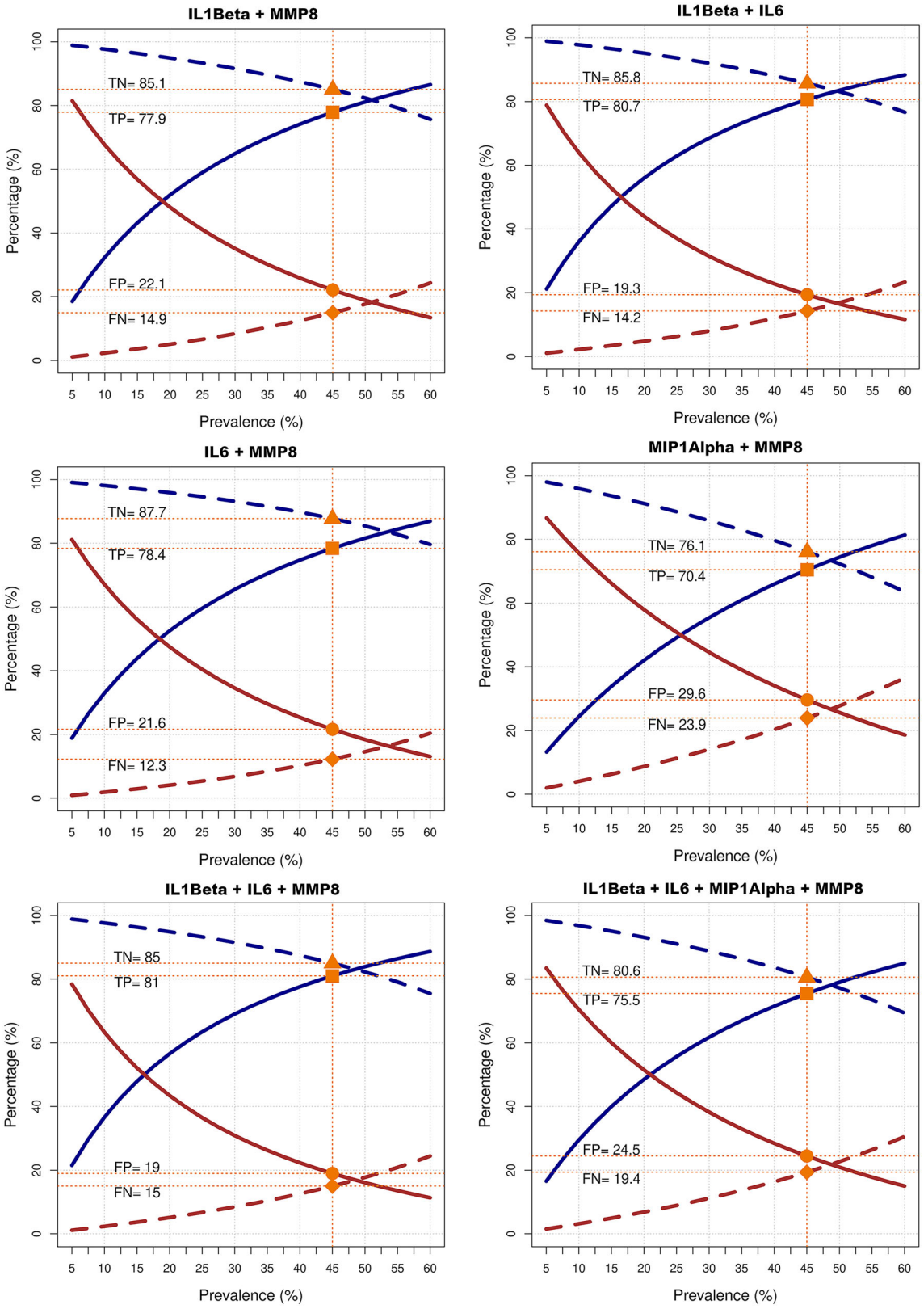

FIGURE 6 Expression of summary accuracy data derived from meta-analyses using natural frequencies based on a hypothetical cohort of 1000 patients along different periodontitis prevalence values for the six combinations of biomarkers most studied in saliva. The continuous lines indicate the percentage of positive tests for different prevalences of periodontitis (the blue line is the percentage of true positives, and the red line is the percentage of false positives). Discontinuous lines indicate the percentage of negative tests for different prevalences of periodontitis (the blue line is the percentage of true negatives, and the red line is the percentage of false negatives). FN, false negative, test is negative (indicates periodontitis not present but the patient has periodontitis); FP, false positive, test is positive (indicates periodontitis but the patient does not have periodontitis); TN, true negative, test is negative (indicates periodontitis not present and the patient does not have periodontitis); TP, true positive, test is positive (indicates periodontitis and the patient has periodontitis). IL, interleukin; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase.

Previously, Baima et al. (2021) had published a systematic review/meta-analysis on GCF metabolites to detect periodontitis. However, only one combination provided diagnostic accuracy (Pei et al., 2020), which did not meet our inclusion criteria. Furthermore, Sukriti et al. (2020) performed a systematic review of the diagnostic accuracy of salivary biomarker combinations, but they did not conduct a meta-analysis due to the heterogeneity and the smaller number of articles detected (7 articles vs. 13 in the present review). The importance of further evidence on the diagnostic accuracy of biomarkers to detect periodontitis was highlighted in both reviews (Baima et al., 2021; Sukriti et al., 2020).

4.1 | Accuracy of GCF biomarker combinations for diagnosing periodontitis

In recent years, high-throughput proteomic techniques have allowed the discovery of new biomarkers that may improve periodontitis diagnosis (Rizal et al., 2020). Applying this approach, the biomarker combinations that demonstrated the highest diagnostic accuracy values were four protein groups formed by 10 biomarkers each and the combination of five proteins (IL-1β, IL-8, MMP-13, OA and OPG) with excellent sensitivity and specificity values above 95% (Baliban et al., 2013; De Luca Canto et al., 2015; Huang et al., 2020). These results are biologically consistent but with limited clinical applicability.

Conversely, Tomás et al. (2017) also showed excellent sensitivity and specificity values (>90%) by combining a pro-inflammatory cytokine (IL-1α, IL-1β and IL-17A) with an anti-inflammatory cytokine (IFN-γ and IL-10). These combinations showed higher diagnostic accuracy than those observed with individual pro-inflammatory cytokines (Tomás et al., 2017). Furthermore, these pro-inflammatory cytokines with the anti-inflammatory cytokine IL-2 as a ratio showed excellent ability to detect periodontitis in non-smokers and smokers, with smokers showing higher sensitivity (>95%) but lower specificity values (Arias-Bujanda et al., 2018). Therefore, tobacco's immunosuppressive effect directly affects cytokines' ability to diagnose periodontitis (Blanco-Pintos et al., 2022).

Accordingly, combinations of a pro-inflammatory cytokine with an anti-inflammatory one could provide effective associations for diagnosing periodontitis, although further studies are needed. However, the low levels of anti-inflammatory cytokines in GCF can make the clinical applicability of these combinations difficult (Tomás et al., 2017).

4.2 | Accuracy of salivary biomarker combinations for the diagnosis of periodontitis

The meta-analysis of six biomarker combinations could be performed in saliva—but not in GCF—although they represented less than 13% of all classification tables.

The association of IL-1β, IL-6 and MMP-8 in pairs showed excellent sensitivity (≥83%) and good specificity (≥81%) (De Luca Canto et al., 2015). However, combining the three proteins did not increase their diagnostic capability (sensitivity/specificity of 82%/84%). The meta-analysis by Arias-Bujanda et al. (2020) showed that IL-1β, IL-6 and MMP-8 individually had sensitivity and specificity values <80% for diagnosing periodontitis. Therefore, combining these proteins in pairs would increase their diagnostic accuracy, with maximum increases of 14% in sensitivity and 13% in specificity.

MIP-1α alone has shown sensitivity and specificity of 65%–68% and 68%–75%, respectively (Ebersole et al., 2015; Görgülü & Doğan, 2022). In this paper, these values increased when this molecule was combined with MMP-8 or IL-1β, IL-6 and MMP-8 (71%–76% and 76%–80%, respectively), although they did not reach the data demonstrated by these biomarkers combined without MIP-1α. Thus, MIP-1α in combination with these proteins might not only increase their diagnostic accuracy but even decrease it.

4.3 | Limitations and strengths

In the present review, the loss of eligible articles was minimized using a search strategy with generic and specific index test terms and was conducted in multiple databases. Additionally, a pre-validated automated data-mining process was used consistent with current trends towards developing automated search systems (Sarker, 2022). However, they can currently reduce sensitivity (Lefebvre et al., 2019).

The main methodological limitation of the included articles is the use of convenience samples to assess biomarker combinations, which may be associated with limited generalizability. Moreover, cross-sectional case–control studies reflect past destruction and present activity but are limited to determining future progression (Jaedicke et al., 2016). The selection of thresholds that provided the highest diagnostic values is justified in the early stages of biomarker discovery. However, the results must be validated to assess the overfitting of predictive models (Moons et al., 2015). In the present review, only 40% of papers on GCF (Arias-Bujanda et al., 2018; Baliban et al., 2013; Huang et al., 2020; Tomás et al., 2017) and 38.5% on saliva (Ebersole et al., 2015; Grant et al., 2022; Kim et al., 2021; Nagarajan et al., 2015; Rzeznik et al., 2017) conducted any validation analysis.

Additionally, variables such as smoking status may influence the diagnostic accuracy of biomarker combinations. Although several articles (30% in GCF and 46% in saliva) included non-smokers and smokers, only three adjusted their results according to this variable (Ochanji et al., 2017; Rzeznik et al., 2017; Tomás et al., 2017). Moreover, only Arias-Bujanda et al. (2018) evaluated the diagnostic accuracy differences in cytokine ratios between non-smokers and smokers. Likewise, previous periodontal treatment is a relevant variable, as it may alter biomarker expression, making it more similar to periodontal health (Ceylan et al., 2022; Keskin et al., 2023). In the present review, considering the studies that provided information on previous periodontal treatment, 50% of articles on GCF and saliva selected patients with never-treated periodontitis. Only one article included patients with periodontitis treated at least 1 month previously (Huang et al., 2020); in another,

the patients were treated during the study (Nakashima et al., 1996). Age differences between target and control groups are also noteworthy (target condition >40 years in ≥70% of GCF and saliva studies; control condition <45 years in 70% of GCF and 62% of saliva studies), as it was found as an influential variable in protein levels (Zhang et al., 2022).

Furthermore, because of the limitations of diagnosing periodontal status based exclusively on the radiographic assessment of BL (Tonetti et al., 2018), this was an exclusion criterion. Although no studies were excluded for this reason, this could have resulted in the loss of eligible studies. Additionally, different clinical definition criteria of periodontal status had to be assumed to make the review feasible. Regardless of the criteria, the reference standard used in 96% of studies was considered adequate, theoretically implying perfect sensitivity/specificity for diagnosing periodontal health or disease (Reistma et al., 2009). However, a perfect reference standard seldom exists (Leeflang & Allerberger, 2019). In periodontitis, since the reference standard measures clinical parameters while the index test considers biological levels of molecular biomarkers, which are unrelated parameters, these inaccuracies may result in underestimating biomarkers' accuracy (Reistma et al., 2009).

Moreover, the heterogeneity of biomarker combinations made it possible to perform a meta-analysis only on six salivary biomarkers. In this regard, it is essential to underline the high number of diagnostic accuracy studies with methodological deficiencies, according to the TRIPOD consortium experts, based on the absence of a binary classification table or adequate information to calculate it (sample size and sensitivity/specificity values) (Moons et al., 2015). Consequently, these papers had to be excluded from this review.

As in other systematic reviews undertaken by our group (Arias-Bujanda et al., 2019, 2020), we considered periodontal health and gingivitis as control conditions, whereas patients with different degrees of periodontitis were selected as target conditions. Similarly, we also considered studies on GCF that evaluated the diagnostic accuracy of biomarker combinations at the site level (Kitamura et al., 1991; Nakashima et al., 1996). For experts in the field, these criteria minimize the overestimation of the diagnostic accuracy of index tests and favour the feasibility of their clinical applicability (Reistma et al., 2009). Nevertheless, it should also be mentioned that this approach might not specifically provide a predictive value for patients with gingivitis.

5 | CONCLUSIONS

5.1 | Implications for clinical practice

Clinical measurements remain the best method for assessing periodontal status (Kinane et al., 2017). However, Tonetti et al. (2018) emphasized that biomarkers could be an essential tool for early diagnosis of periodontitis. GCF and saliva are the most used, as they can be easily and non-invasively collected (Guzman et al., 2014) and their analyses are cost effective for detecting biomarkers (Zhang et al., 2009). Moreover, because of advances in biomarker detection technologies, point-of-care periodontitis diagnosis is feasible (Sorsa et al., 2022). This strategy can be useful as an automatic, self-performing screening tool (e.g., using smart toothbrushes) to enable patients themselves or general clinicians to interpret the result and refer them to a dentist (Bornes et al., 2023).

However, owing to the aetiopathogenic complexity of the disease, it seems highly unlikely to find a single biomarker that allows accurate diagnosis (Bibi et al., 2021; Zhang et al., 2009). Combining more than one biomarker could provide a more precise assessment of the patient's periodontal status (Ghallab, 2018). Interestingly, none of the 68 biomarker combinations evaluated coincided in both oral fluids. This reflects that scientific research is more focused on discovering new combinations only in saliva rather than identifying biomarker combinations in GCF and then validating them in saliva (Cafiero et al., 2021).

Considering clinical applicability, the best combination of biomarkers would be formed by the least number of molecules possible, that is, two biomarkers with high diagnostic accuracy. In this regard, the present review revealed that incorporating more biomarkers did not improve the diagnostic accuracy of periodontitis more significantly than when using the best combinations of two biomarkers in oral fluids. Focusing on the most investigated salivary two-biomarker combinations and considering a 45% prevalence of periodontitis in different stages (Eke et al., 2020; Tonetti et al., 2015), the use of a first-line screening tool IL-1β with MMP-8, IL-1β with IL-6 or IL-6 with MMP-8 would correctly identify 78%–81% of periodontal patients and 85%–88% of periodontally healthy subjects. These results did not improve by combining these three biomarkers. These findings were obtained in cross-sectional studies and, therefore, indicate the accuracy of these biomarker combinations in detecting already developed periodontitis. Consequently, they do not reflect their potential ability to predict the future development or progression of periodontitis.

5.2 | Implications for future research

Based on the evidence reviewed, more high-quality research is needed on the accuracy of biomarker combinations for diagnosing periodontitis in oral fluids, especially GCF. These studies should have large, age-matched samples, assess the impact of smoking and periodontal treatment and include validation analyses (Moons et al., 2015). Similarly, there is a need for more methodologically well-designed longitudinal investigations that evaluate the prognostic ability of biomarkers in periodontitis. This study design can allow the discovery of biomarkers that can predict the development of periodontitis or anticipate its progression to severe stages. In this regard, techniques such as high-throughput proteomics have proven to be valuable tools for identifying new and more accurate biomarkers (Van Gool et al., 2020). Also, the meta-analytic and network-type approaches available for therapeutic questions should be extended to questions of comparative diagnostic or prognostic accuracy (Leeflang & Reitsma, 2018).

In conclusion, dual combinations of some biomarkers in oral fluids show high diagnostic accuracy for periodontitis, which is not substantially improved by incorporating more biomarkers. In GCF, pro-

inflammatory and anti-inflammatory cytokines combine well to diagnose periodontitis. Based on the meta-analytical results obtained in saliva, dual combinations of IL-1β, IL-6 and MMP-8 show excellent ability to detect periodontitis and good capacity to detect non-periodontitis. However, because of the heterogeneity and the limited number of biomarker combinations evaluated, further evidence, mainly in GCF, is needed to corroborate these findings.

AUTHOR CONTRIBUTIONS

C. Balsa-Castro and I. Tomás contributed to the conception and design of the study. A. Regueira-Iglesias, T. Blanco-Pintos and I. Seijo-Porto performed the literature search, selected the articles of interest and extracted the relevant information. P. Castelo-Baz performed the analysis of the methodological quality of the studies. C. Balsa Castro performed the computerized literature search and the meta-analysis. A. Regueira-Iglesias, T. Blanco-Pintos and C. Balsa-Castro made the graphs, tables and additional files. T. Blanco-Pintos, I. Seijo-Porto and I. Tomás drafted the manuscript. P. Castelo-Baz and L. Nibali carried out a critical revision of the manuscript and gave comments for improvement. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

The authors ensure that all research is conducted in accordance with ethical principles.

ORCID

P. Castelo-Baz https://orcid.org/0000-0003-3031-5532
L. Nibali https://orcid.org/0000-0002-7750-5010
I. Tomás https://orcid.org/0000-0002-3317-0853

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