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REVIEW ARTICLE

Microbial biomarkers as a predictor of periodontal treatment response: A systematic review

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Abstract

To evaluate the prognostic accuracy of microbial biomarkers and their associations with the response to active periodontal treatment (APT) and supportive periodontal therapy (SPT). Microbial dysbiosis plays a crucial role in the disease processes of periodontitis. Biomarkers based on microbial composition may offer additional prognostic value, supplementing the limitations of current clinical parameters. While these microbial biomarkers have been clinically evaluated, there is a lack of consensus regarding their prognostic accuracy. A structured search strategy was applied to MEDLINE (PubMed), Cochrane Library, and Embase on 1/11/2022 to identify relevant publications. Prospective clinical studies involving either APT or SPT, with at least 3-month follow-up were included. There were no restrictions on the type of microbial compositional analysis. 1918 unique records were retrieved, and 13 studies (comprising 943 adult patients) were included. Heterogeneity of the studies precluded a meta-analysis, and none of the included studies had performed the sequence analysis of the periodontal microbiome. Seven and six studies reported on response to APT and SPT, respectively. The prognostic accuracy of the microbial biomarkers for APT and SPT was examined in only two and four studies, respectively. Microbial biomarkers had limited predictive accuracy for APT and inconsistent associations for different species across studies. For SPT, elevated abundance of periodontal pathogens at the start of SPT was predictive of subsequent periodontal progression. Similarly, persistent high pathogen loads were consistently associated with progressive periodontitis, defined as an increased pocket probing depth or clinical attachment loss. While there was insufficient evidence to support the clinical use of microbial biomarkers as prognostic tools for active periodontal treatment outcomes, biomarkers that quantify periodontal pathogen loads may offer prognostic value for predicting progressive periodontitis in the subsequent supportive periodontal therapy phase. Additional research will be required to translate information regarding subgingival biofilm composition and phenotype into clinically relevant prognostic tools.

1 | INTRODUCTION

Periodontitis is a prevalent complex, biofilm-induced, chronic inflammatory disease of the tooth-supporting structures that is also influenced by environmental, behavioural, and systemic factors.[1] Half the adult population presents with at least some form of periodontal disease and severe periodontitis affects 11.2% of the adult population,[2] highlighting significant variability in patient disease susceptibility. This is further complicated by tooth- and site-specific factors, such as tooth position, morphology, and dentoalveolar relationship, leading to additional intra-patient variability in disease severity.[3] In addition, the response to active periodontal treatment (APT), and the risk for relapse during the subsequent supportive periodontal therapy (SPT), can also vary at patient, tooth, and site level, translating to substanstially variable treatment needs within the population. Adopting a personalised medicine approach in managing periodontitis can shift the focus towards earlier detection and interceptive treatment for susceptible individuals, preventing the downstream complications of periodontitis.[4] To deliver such personalised and interceptive treatment, the ability to predict the risk of developing periodontitis, its progression, and treatment response will be critical.

Currently, the diagnosis and monitoring of periodontitis rely on clinical parameters such as pocket probing depths (PPDs), clinical attachment level (CAL), and bleeding on probing (BOP).[5] These parameters are also used as a benchmark for treatment planning, prognostication, and as an endpoint for active treatment.[6] While these clinical parameters have a high negative predictive value,[7,8] it is well established that they are a measure of disease history as opposed to current disease process.[9] As such, clinical parameters do not accurately identify patients or sites that are susceptible to periodontitis, nor do they predict the disease trajectories and response to treatment. These limitations have resulted in a passive approach, where treatment is rendered only after periodontitis has occurred and manifested with deepened PPDs, hindering the implementation of personalised and proactive periodontal care. To address this clinical problem, there is a need to develop effective prognostic tools that are sensitive to the underlying disease processes of periodontitis to complement existing clinical parameters.

From periodontal health to periodontitis, a change in the host-biofilm relationship from symbiosis to dysbiosis occurs, with concomitant changes in the composition of the subgingival microbiome.[10] In light of the microbial aetiology of periodontitis, biomarkers based on the microbial composition may provide insight into the biological status of the periodontal pocket. Furthermore, the success of periodontal treatment is highly dependent on the effective disruption and removal of the subgingival biofilm.[11] As such, the microbial composition prior to and after treatment may provide prognostic value on the short-term outcomes following APT and long-term stability during SPT. Although the diagnostic value of microbial biomarkers has been previously appraised,[12] there is no consensus regarding their prognostic value. Therefore, the aim of this systematic review was to evaluate the prognostic accuracy of microbial biomarkers and their associations with the response to periodontal treatment.

2 | MATERIALS AND METHODS

2.1 | Protocol development

This systematic review was designed and conducted according to the Cochrane Handbook for Systematic Reviews of Interventions,[13] and reported according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement,[14] to answer the following focused question; "In prospective clinical studies involving periodontitis patients, are microbial biomarkers predictive of, or associated with, the outcomes of active and supportive periodontal therapy?" The study protocol was registered in the "International Prospective Register of Systematic Reviews" (CRD42022371864).

2.2 | Eligibility Criteria

2.2.1 | Inclusion criteria

- Population: Prospective clinical studies conducted in the dental setting, involving patients receiving treatment for periodontitis. There were no restrictions on the participant characteristics (age, gender, ethnicity, tobacco smoking), severity or type of periodontitis, or the type of periodontal treatment.
- Prognostic test: Microbial compositional analysis of salivary, supra- or subgingival biofilm samples obtained before and/or after active periodontal treatment. There were no restrictions on the approach used for microbial analysis.
- Outcomes: Patient- or site-level response, to active periodontal treatment or supportive periodontal therapy (APT and SPT, respectively), defined using periodontal parameters.

2.2.2 | Exclusion criteria

- Studies involving patients with gingivitis or peri-implant disease.
- Studies that did not involve professionally rendered periodontal treatment.
- Studies without a minimal follow-up of 3 months after APT or 1 year after SPT.

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- Studies reporting on disease progression in untreated periodontitis.
- Studies that did not define treatment response or outcomes using periodontal parameters or studies that only report the impact on tooth loss or tooth survival.
- Studies that did not report the predictive value of, nor association with, the subsequent outcomes of APT or SPT.
- In vivo or in vitro studies, reviews, expert opinion, guidelines, and conference abstracts.
- Studies published in languages other than English.

2.3 | Information sources and search strategy

The PRISMA statement and flow diagram was utilised in this review, and a search protocol was developed a priori following a preliminary search and discussion between members of the research team. A structured electronic search was conducted on 1/11/2022, involving the following electronic databases; Cochrane Library, MEDLINE (PubMed), and Embase. The search terms used consisted of keywords and Mesh terms connected with Boolean operators, encompassing 3 key concepts, microbial biomarkers, periodontal therapy, and treatment outcomes. The detailed search strategy for each electronic database is summarised in Table SS1. To identify any additional eligible studies, a hand search of the reference lists of the included studies was performed.

2.4 | Screening and selection

The search results from each database search were imported into EndNote reference management software (Endnote version X9.3.1 Clarivate Analytics) to merge the search results and remove duplicate records. Two calibrated reviewers (RJJC and CEG) then independently carried out the title and abstract screenings based on the described eligibility criteria, and agreement between reviewers was evaluated using Cohen's kappa. Subsequently, full-text articles were retrieved and reviewed for inclusion in this review. Any potential disagreements or discrepancies were resolved with discussion. The number of excluded articles at each stage is recorded in the PRISMA flow chart (Figure 1).

2.5 | Data extraction

Data extraction was performed by two independent reviewers (RJJC and CEG), using a piloted data extraction form. The extracted data included the following:

- Author, title, publication details, and study design.
- Patient characteristics, including demographic information (age and gender), periodontal parameters, presence of known periodontal risk factors (tobacco smoking, systemic diseases), and diagnostic criteria for periodontitis.

Identification of studies via databases and registers

| Identification | Records identified from (n = 2624): MEDLINE (PubMed) (n = 1480) Embase (n = 745) Cochrane Library (n = 399) | → | Records removed *before screening*: Duplicate records removed (n = 706) |

Records screened (n = 1918) → Records excluded (n = 1863)

Reports sought for retrieval (n = 55) → Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 55) → Reports excluded:
- Quantitative measures of prognostic value or association with subsequent treatment outcomes not reported (n = 33)
- No periodontal treatment rendered (n = 2)
- Cross sectional study (n = 2)
- Inadequate follow-up post-treatment (n = 3)
- Conference abstract (n = 1)

Studies included in review (n = 13) Reports of included studies (n = 17)

Identification of studies via other methods

Records identified from: Citation searching (n = 21)

Reports sought for retrieval (n = 18) → Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 18) → Reports excluded (n = 15): Quantitative measures of prognostic value or association with subsequent treatment outcomes not reported (n = 11) No Periodontal treatment rendered (n = 4)
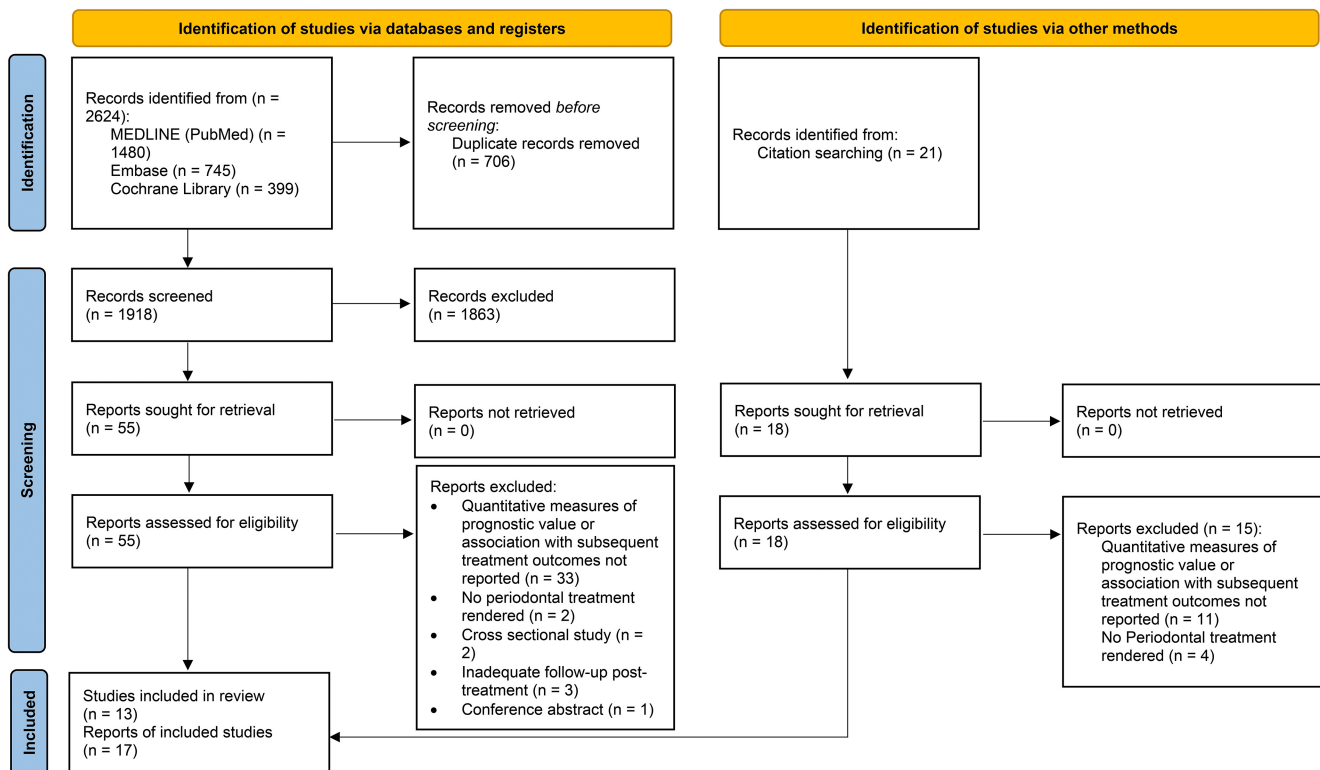
FIGURE 1 PRISMA flow diagram.

TABLE 1 Summary of the descriptive characteristics of the 13 included studies.

Author (year)	Study design	Country	Inclusion criteria for periodontitis	Follow-up
Brochut et al (2005)	Prospective cohort	Switzerland	Moderate to advanced periodontitis with at least 4 teeth with PPD ≥6mm	6m
Byrne et al (2009)	Prospective cohort	Australia	Previously diagnosed with chronic periodontitis, had completed their treatment and had been on a maintenance program for a minimum of 6months	12m
Charalampakis et al (2013)	Prospective cohort	Sweden	Advanced periodontal disease, with at least 1 diseased (PPD ≥6mm with BOP) and 1 healthy site (PPD ≤3mm without BOP)	24m
Colombo et al (1998)	Prospective cohort	USA	At least 8 sites with PPD >4mm and attachment level >3mm	12m
Čuk et al (2020)	Randomised control trial	Slovenia	Untreated moderate to advanced periodontitis with PPD ≥5mm at a minimum of 4 teeth in 4 different quadrants	6m
Eick et al (2017)	Prospective cohort	Germany	Moderate to severe chronic periodontitis with at least 20 sites with PPD ≥4mm	6m
Gul et al (2017)	Prospective cohort	UK	A diagnosis of chronic periodontitis with diseased sites	6m
Heitz-Mayfield et al (2006)	Randomised control trial	Belgium, Germany, Greece, Italy, Switzerland, UK, USA	A diagnosis of severe periodontitis previously treated by oral hygiene instructions and scaling and root planning	12m
Kakuta et al (2017)	Prospective cohort	Japan	Individuals with chronic periodontitis who had completed initial therapy or periodontal surgery	24m
Keyes et al (2015)	Prospective cohort	USA	Moderate to severe periodontitis	4.5±1.0[a] years
Mombelli et al (2017)	Randomised control trial	Switzerland	Moderate-to-advance periodontitis with at least 4 teeth showing radiographic evidence of bone loss, clinical attachment loss ≥2mm and PPD >4mm at one or several sites	12m
Nomura et al 2012	Prospective cohort	Japan	Chronic periodontitis with 2 or 3 sites with PPD ≥5mm after active periodontal treatment	18m
Rams et al (1996)	Prospective cohort	USA	Moderate to advanced adult periodontitis patients with a history of recurrent disease activity	12m

Abbreviations: PPD, pocket probing depth; NA, not applicable; NR, not reported; BOP, bleeding on probing; CP, chronic periodontitis; T1, Nonsurgical root debridement; T2 Open flap debridement.

[a]Excludes dropouts.

[b]Median value (25–75 percentile).

- Details pertaining to periodontal treatment (type of APT and/or SPT and the length of follow-up).
- Details pertaining to the methodology employed for microbial sampling and analysis.
- Criteria used to assess the patient and/or site response to periodontal treatment.

Data on the periodontal and microbial outcome measures were extracted for the baseline and for all reported post-treatment time points. If these outcomes were only reported graphically in figures, validated software (WebPlotDigitizer, Pacifica) was used to extract the data.[15] In the event of incomplete or missing data, attempts were made to contact the corresponding author of the included studies

Groups	Type of periodontal treatment	Age (mean ± SD)	Sample size	Gender (male/female)	Smokers	Dropouts
NA	Nonsurgical root debridement	45 ± 8.7 years	10	6/4	0 smokers	0
NA	Supportive periodontal therapy	60.1 ± 11.2 years	41	13/28	NR	0
NA	Supportive periodontal therapy	54.6 ± 11.2 years	50	20/30	23 current, 4 former smokers, 3 snuff	15
Successfully treated patients	Nonsurgical root debridement, followed by modified Widman flap surgery and adjunctive tetracycline	49 ± 1.4 years	66	33/33	15% current, 49% former smokers	0
Refractory patients		45 ± 2.3 years	28	16/12	23% current, 42% former smokers	0
Test group	Nonsurgical root debridement with adjunctive azithromycin	45.4 ± 10.5 years	20	12/8	4 smokers	1
Control group	Nonsurgical root debridement	44.0 ± 8.5 years	20	14/6	5 smokers	1
NA	Nonsurgical root debridement	55.4 ± 9.8	46	21/25	19 smokers	0
NA	Nonsurgical root debridement	49.7 ± 8.9 years	89	44/45	8 smokers	12
Test group	Guided tissue regeneration	49.5 ± 11.3 years[a]	61	24/34[b]	21 smokers[b]	3
Control group	Access flap with papilla preservation	51.0 ± 10.5 years[a]	59	22/36[b]	19 smokers[b]	1
Stable CP patients	Supportive periodontal therapy	60 years (56–66 years)[a,b]	163	23/39[b]	3 smokers[b]	39
Progressive CP patients		61 years (52.8–68 years)[a,b]		26/36[b]	1 smoker[b]	
NA	Supportive periodontal therapy	47.3 ± 9.8 years	47	14/33	6 smokers	0
Protocol A	Adjunctive amoxicillin and metronidazole during T1	45.7 ± 8.3 years	40	19/21	16 smokers	2
Protocol B	Adjunctive amoxicillin and metronidazole during T2	48.9 ± 9.1 years	40	22/18	17 smokers	7
Non-progressive chronic periodontitis patient	Supportive periodontal therapy	60.2 ± 10.2 years	28	7/21	2 smokers	0
Progressive chronic periodontitis patient		60.1 ± 6.5 years	57	15/42	18 smokers	0
Disease active subjects	Supportive periodontal therapy	57.1 ± 2.4 years	25	17/8	7 current smokers	0
Clinically stable subjects		56.5 ± 1.9 years	53	24/29	7 current smokers	0

for clarification. Tables were subsequently created to summarise the above items.

2.6 | Outcome measures and synthesis of the results

The primary outcomes of this review were the sensitivity, specificity, and positive and negative predictive values of microbial biomarkers used to predict response to periodontal treatment. In addition, Youden's index for each microbial biomarker was calculated using the reported sensitivity and specificity. For the secondary outcomes, the association between the biomarkers and treatment response was summarised using odds ratios (OR), risk ratios (RR), or hazard ratios (HR), with their respective 95% confidence intervals. Considering the differences in study design, types of periodontal treatment, treatment outcome definitions, microbial sampling, analytical methodology, and different microbial

TABLE 2 Prognostic accuracy measurements of microbial biomarkers.

Author (year)	Definition for treatment outcome	Sampling methodology	Timepoint	Detection method
Brochut et al (2005)	Site-level clinical success defined as PPD <5 mm without BOP	Paper points, full mouth sampling from the deepest pocket of each tooth	Week 6 after treatment	DNA hybridisation probe
Charalampakis et al (2013)	Site-level disease progression defined as ≥2 mm increase in PPD with CAL ≥2 mm, between the 2 follow-up timepoints (2006 and 2008)	Paper points, subgingival biofilm sampled from one diseased site (PPD ≥6 mm with BOP) and one clinically healthy/gingivitis/mild periodontitis site (no BOP or BOP and PPD up to 4 mm)	After nonsurgical therapy and 2 yr of maintenance	Checkerboard DNA–DNA hybridisation
Kakuta et al (2017)	Patient-level periodontitis progression defined as at least 1 site exhibiting CAL ≥3 mm during the 24-month study period	Paper points, subgingival biofilm sampled from the deepest pockets of each patient	Baseline	Invader PLUS assay, a PCR-based technique
Mombelli et al (2017)	Patient-level treatment outcome defined as presence of at least 1 site with PPD >4 mm and BOP, 12 months post-therapy	Paper points, pooled subgingival biofilm from the deepest PPD from each quadrant	Baseline	Quantitative PCR
Nomura et al (2012)	Patient-level outcome defined by at least one site with progression of CAL of >3 mm	Whole stimulated saliva	Baseline	Quantitative PCR
Rams et al (1996)	Patient-level outcome defined as recurrent periodontitis at 6 and 12 months characterised by a PPD increase of ≥3 mm from baseline, or PPD increase ≥2 mm together with a CAL ≥2 mm from baseline	Paper points, pooled subgingival biofilm from deepest pocket of each sextant	Baseline	Anaerobic culture

Abbreviations: PPD, pocket probing depth; BOP, bleeding on probing; NR, not reported; CAL, clinical attachment loss; PCR, polymerase chain reaction.

[a]Denotes that the associations were not statistically significant.

[b]Presented results calculated from the data reported by each study.

[c]Red complex consists of *P. gingivalis*, *T. forsythia* and *T. denticola*.

[d]Complex A consists of *P. tannerae*, *F. alocis*, and *P. endodontalis*.

[e]Complex B consists of *P. intermedia*, *F. nucleatum* and *C. rectus*.

species analysed, a meta-analysis was not feasible. Instead, a narrative synthesis was performed. The studies were categorised according to their treatment phase (APT or SPT) and then subcategorised according to the outcomes reported (predictive value and associations).

2.7 | Risk of bias in individual studies

Two reviewers (RJJC and CEG) independently assessed the included studies for risk of bias using the Quality Assessment of Prognostic Accuracy Studies (QUAPAS) tool.[16] Briefly, the QUAPAS tool is an

Microbial biomarker outcome definitions	Microbial biomarker	Sensitivity/ Specificity	PPV/NPV	Youden's Index[b]
Positive test result defined as the absence of the target microbe	A. actinomycetemcomitans	0.35/0.57	NR	−0.08
	P. gingivalis	0.18/0.60	NR	−0.22
A high checkerboard score was defined as a score of 3 ($>10^5$ bacteria) or more, representing heavy colonisation	At least one "red complex[c]" species with a high score	0.80/0.89	NR	0.69
	At least two "red complex" species with a high score	0.80/0.91	NR	0.71
	At least one "complex A[d]" species with a high score	0.79/0.74	NR	0.54
	At least two "complex A" species with a high score	0.80/0.86	NR	0.65
	At least one "complex B[e]" species with a high score	0.79/0.77	NR	0.57
	At least two "complex B" species with a high score	0.60/0.90[a]	NR	0.50
Positive test result when the P. gingivalis, P. intermedia and A. actinomycetamcomitans counts ($\log_{10}$) exceeds 1.370, 1.040 and 1.151 respectively	P. gingivalis	0.55/0.77	0.71/0.63	0.32
	P. intermedia	0.37/0.77[a]	0.62/0.55	0.15
	A. actinomycetemcomitans	0.07/0.98[a]	0.80/0.51	0.05
Positive test defined as >1000 cells/mL at baseline	P. intermedia	0.84/0.41	NR	0.25
	P. micra	0.84/0.37	NR	0.21
Positive test when the P. gingivalis, P. intermedia and T. forsythia counts ($\log_{10}$) exceed 4.7, 5.2 and 4.8 respectively	P. gingivalis	0.68/0.68	0.81/0.51	0.36
	P. intermedia	0.70/0.68	0.82/0.53	0.38
	T. forsythia	0.58/0.54[a]	0.72/0.39[a]	0.12
Positive test when the P. gingivalis, P. intermedia and T. forsythia ratio (%) exceed 0.031, 0.017 and 0.013 respectively	P. gingivalis	0.68/0.68	0.81/0.51	0.36
	P. intermedia	0.67/0.64	0.79/0.49	0.31
	T. forsythia	0.58/0.57[a]	0.73/0.40[a]	0.15
Positive test result defined by the presence of ≥1 bacterial species (A. actinomycetemcomitans, P. gingivalis, P. intermedia, C. rectus, or P. micros)	≥1 bacterial species present (6 months)	1.00/0.18	0.22/1.00	0.18
	≥1 bacterial species present (12 months)	0.88/0.15	0.32/0.73	0.03
Positive test result defined by ≥1 bacterial species recovered at or above the threshold proportions of ≥0.01% for A. actinomycetemcomitans, ≥0.1% for P. gingivalis, ≥2.5% for P. intermedia, ≥2.0% for C. rectus, ≥3.0% for P. micros	≥1 bacterial species recovered at or above the threshold proportions (6 months)	0.80/0.43	0.25/0.90	0.23
	≥1 bacterial species recovered at or above the threshold proportions (12 months)	0.80/0.47	0.42/0.83	0.27

adaptation of the Quality Assessment of Diagnostic Accuracy Studies 2 tool, modified to address the risk of bias that arises from the longitudinal study design of prognostic studies. The tool encompasses five domains, the participants, index test, outcome, flow and timing, and the analysis. Each domain was graded as either high, low, or unclear risk of bias. Additionally, concerns regarding the applicability of the findings of each study to the focused question of this review were graded as high low, or unclear in the context of the first 4 domains. In the event clarification was necessary, attempts were made to contact the corresponding author of the included studies. In cases of disagreements, the overall risk of bias assessments was resolved by consensus following discussions between the two reviewers.
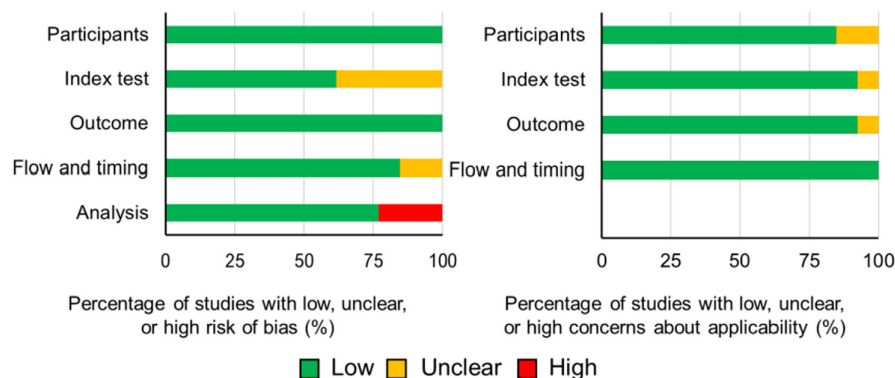
FIGURE 2 Summary of the risk of bias and applicability judgements for the microbial biomarkers.

3 | RESULTS

The systematic review process is summarised in the PRISMA flow-chart (Figure 1). The electronic search yielded a total of 1918 unique records. 1863 records were excluded during the title and abstract evaluation (inter-examiner agreement of $\kappa=0.92$). Of the 55 reports retrieved for full-text evaluation, 41 reports were excluded due to the following reasons: (i) quantitative measures (i.e., sensitivity/specificity, or odds ratios) of the prognostic value or association with subsequent treatment outcomes were not reported, (ii) lack of professionally rendered periodontal treatment, (iii) cross-sectional study design, (iv) an inadequate post-treatment follow-up of <3 months after APT, or <1 year after SPT, and (v) conference abstract (Figure 1, Table S2). An additional 3 reports were identified during the hand search, and a total of 13 studies were included in this review.[17–33]

3.1 | Study characteristics

The characteristics of the included studies, published from 1996 to 2020, are summarised in Table 1. Of the 13 included studies, three randomised controlled trials evaluated the association between microbial biomarkers and the outcomes of APT as secondary outcomes. In the remaining 10 prospective cohort studies, the implications of microbial biomarkers on APT and SPT outcomes were the primary outcome reported. The 13 studies involved a total of 943 adult patients with a diagnosis of periodontitis. While the majority (11 studies) excluded patients with systemic diseases or medications that may influence periodontal health and therapy, 50% of the population in one study presented with health conditions such as diabetes mellitus.[19] Details pertaining to the systemic health of the included patients were not reported one study.[29] A varying number of smokers were included in 11/13 studies, whereas one study only included non-smokers.[17] In the remaining study, smoking status of the included individuals was not reported.[18]

While most of the included studies collected subgingival biofilm using sterile curettes (5 studies) or paper points (7 studies), one study evaluated the microbial composition of whole unstimulated saliva.[28] The composition of the subgingival biofilm was generally analysed with a specific microbe approach, with 10 studies employing molecular techniques such as polymerase chain reaction (PCR), or DNA hybridisation probe, while 2 studies employed anaerobic culture.[21,29] One study evaluated the subgingival biofilm samples using phase-contrast microscopy, quantifying the microbes based on their morphology.[26] None of the included studies employed next-generation sequencing analysis of the microbiome composition. The majority of the evaluated microbes were established periodontal pathogens including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsynthia*, *Treponema denticola*, *Prevotella intermedia*, *Parvimonas mirca*, *Fusobacterium nucleatum*, *Streptococus constellatus*, *Campylobacter rectus*, and *Peptostreptococcus micros*. Novel pathogens such as *Filifactor alocis*, *Prevotella tannerae*, and *Porphyromonas endodontalis* were also evaluated by one study.[19]

3.2 | Risk of bias analysis

The results of the risk of bias assessment, using QUAPAS are summarised in Figure 2. In general, the risk of bias and concerns about applicability were low. For the conduct of the index test, 5 studies were deemed to have an unclear risk of bias as they did not predefine the threshold cut-offs used to dichotomise the microbial test results.[19,20,26,28,29] For the study flow and timing, due to the variable SPT intervals,[19] and differential treatment of patients who were described (as per previous classification systems) as having refractory periodontitis,[20] two studies were found to have an unclear risk of bias. Lastly, 3 studies were found to have a high risk of bias for their analysis, due to the lack of appropriate measures to account for their significant patient dropout.[19,23,25]

For three studies, the applicability of some domains to the current review question was judged as unclear. One study recruited a significant proportion of patients who were classified as being refractory to prior treatment,[20] representing a population that is at a higher risk for poor treatment response, which may not apply to the general population of periodontitis patients. In another, favourable treatment outcomes were defined as at least 60% reduction in the number of sites with PPD >4 mm,[22] which may be an arbitrary threshold that also differs from the established outcome measures such as mean changes in PPD or CAL, or the number of residual PPDs deeper than 4 or 5 mm.[6] Thus, their study findings may not be

generalisable to more common clinically relevant outcomes. The last study employed unconventional periodontal treatments and a microbial assessment based on phase-contrast microscopy,[26] which provides limited and non-specific insight into the biofilm composition.

3.3 | Narrative synthesis

3.3.1 | Implications of microbial biomarkers on APT

The 7 included studies evaluated microbial biomarkers predicting or associated with treatment response following a range of active periodontal therapies, including nonsurgical root debridement, with or without adjunctive antibiotics, access flap surgeries, and periodontal regeneration (Table 1). These studies had a follow-up of 6–12 months.

The primary outcome of predictive accuracy was reported by only two studies.[17,27] Absence of *A. actinomycetemcomitans* and *P. gingivalis* from a site deeper than 5 mm 6 weeks after root debridement, had a sensitivity of 0.35 and 0.18, respectively, for predicting a successful outcome (characterised as a post-treatment PPD of <5 mm without BOP) at 6 months[17] (Table 2). In contrast, the persistence of *A. actinomycetemcomitans* and *P. gingivalis* at 6 weeks could only accurately predict 57% and 60% of the sites with unsuccessful treatment outcomes. Despite achieving statistical significance, these biomarkers were found to have limited clinical utility due to their low predictive accuracy. In the second study, a high microbial load of *P. intermedia* and *P. mirca*, prior to treatment were both found to be predictive of residual deep pockets (at least 1 site with PPD >4 mm) after active treatment, each with a high sensitivity of 0.84. However, both bacteria were found to have a low specificity, 0.41 and 0.37, respectively, limiting their overall predictive accuracy, evidenced by their respective Youden's index values of 0.25 and 0.21.

The secondary outcomes were reported in 6 studies, quantifying the associations between the microbial biomarkers and treatment outcomes (Table 3).[20–24,27] Although the rendered APT was variable, these studies can be broadly classified as those that evaluated the presence of specific pathogens or those that evaluated their microbial load. In the former, significant associations were generally not observed for both patient-level[22] and site-level[21] outcomes following nonsurgical periodontal therapy. On the other hand, when the abundance of these pathogens was analysed, a positive test result for *P. gingivalis*, *T. forsynthia*, *T. denticola*, *P. intermedia*, *P mirca*, and *S. constellatus* was found to be statistically significantly associated with inferior clinical outcomes following both nonsurgical periodontal therapy[14,23,27] and periodontal surgery.[20,24] Notably, for both access flap surgeries and periodontal regeneration, the total microbial abundance and the abundance of *T. forsynthia* alone, or in combination with *P. gingivalis* and *T. denticola* exhibited a negative dose-dependent effect on CAL gain.[24] These findings suggest that a critical mass of pathogens is required to elicit an effect on the outcomes of APT.

However, while several pathogens were repeatedly evaluated, individual studies reported significant associations for different species. To illustrate the point, while *P. gingivalis* was identified to have some predictive value,[17] and the presence or abundance of *P. gingivalis* was analysed in 5 out of 6 studies, only one study reported a significant association with treatment outcomes.[23] Thus, the exact implications of this microbial biomarker on APT remain inconclusive due to methodological heterogenicity and a lack of consistent results.

3.3.2 | Implications on SPT

Six studies evaluated the implications of microbial biomarkers implications on periodontal disease progression defined as either additional CAL loss or increased PPDs during SPT. Elevated scores for spirochaetes alone, or coupled with medium and large motile rods at the start of SPT were significantly associated with progressive periodontal disease during a SPT period of 4.5 ± 1 years.[26] Similar to our observations with APT, while the presence of specific pathogens (*A. actinomycetemcomitans* and *P. gingivalis*, *P. intermedia*, *C. rectus*, or *P. micros*) was not significantly associated with periodontitis progression, increased abundance of at least one of these microbes in the deepest pockets of all 6 sextants was associated with periodontal disease progression at the patient-level.[29] Similarly, at the patient level, the abundance of *P. gingivalis* in sites of deepest PPDs was also associated with periodontal progression during 24 months of SPT.[25] At the site level, an increased microbial load for *P. gingivalis* and *T. forsythia*, but not *T. denticola*, was associated with disease progression 3 months later.[18] In the last study, a broad range of microbial species was categorised into 3 complexes, namely the red complex (*P. gingivalis*, *T. forsythia* and *T. denticola*), Complex A (*P. tannerae*, *F. alocis*, and *P. endodontalis*) and Complex B (*P. intermedia*, *F. nucleatum* and *C. rectus*).[19] A high microbial load of at least one or two of the species of each complex, at the monitored sites, was significantly associated with periodontal relapse, quantified as odds ratios that ranged from 12.25 to 61.00. The odds ratios reported in this study have wide confidence intervals, reflecting the imprecision of the results (Table 3) which may have resulted due to a sparse data bias.[34] Furthermore, this study was also identified to have a high risk of bias, having failed to account for the loss to follow-up in an already small sample size. Nevertheless, the results of these studies support the notion that the failure to adequately suppress the microbial load of periodontal pathogens after APT was associated with relapse and progressive periodontitis during SPT.

Similar observations were reported by the 4 studies evaluating the predictive accuracy of the microbial biomarkers for disease progression during SPT. Although the presence of 5 pathogens was highly sensitive (0.88 and 1.00 at 6 and 12 months, respectively) for disease progression, the specificity was low (0.15 and 0.18 at 6 and 12 months, respectively) limiting their prognostic value.[29] In contrast, when a threshold value was applied for the microbial abundance, while the sensitivity was reduced to 0.80, the specificity was improved to 0.43 and 0.47 at 6 and 12 months, respectively. Similarly, elevated counts and proportions

TABLE 3 Association between microbial biomarkers and treatment outcomes.

Author (year)	Definition for treatment outcome	Sampling methodology	Timepoint
Byrne et al (2009)	Site-level periodontal disease progression defined as an increase in CAL ≥2mm during the study	Curette, subgingival biofilm sampled from 5 deepest sites that were anterior to and including the mesial surface of the first molar	During supportive periodontal therapy (3 monthly interval)
Charalampakis et al (2013)	Site-level disease progression defined as ≥2mm increase in PPD with CAL ≥2mm, between the 2 follow-up timepoints (2006 and 2008)	Paper points, subgingival biofilm sampled from one diseased site (PPD ≥6mm with BOP) and one clinically healthy/gingivitis/mild periodontitis site (no BOP or BOP and PPD up to 4mm)	After nonsurgical therapy and 2yr of supportive periodontal therapy
Colombo et al (1998)	Patient-level disease progression defined as either mean CAL, as determined by full mouth attachment level measurements taken pre- and post-therapy and/or more than 3 sites with CAL >2.5mm within a period of 1 year	Curette, subgingival biofilm sampled from the mesio-buccal aspect of all teeth, except the 3rd molars	Baseline
Čuk et al (2020)	Site-level healing of diseased sites (PPD ≥5mm with BOP) defined as (1) BOP and PPD <5mm and/or (2) no BOP	Paper points, pooled subgingival microbes sampled from the 4 sites in each jaw quadrant, with the deepest PPD	Baseline
Eick et al (2017)	Patient-level treatment response classified as high or low response to treatment based on a cut-off of ≥60% reduction of sites with PPD >4mm, 6months post-treatment	Paper point, subgingival biofilm sampled from the deepest sites of each quadrant	Baseline and 3-month post-treatment
Gul et al (2017)	Site-level responsiveness to treatment for sites defined as PPD reduction of ≥2mm compared to baseline	Curette, subgingival biofilm sampled from 3 representative sites: (1) PPD ≥6mm with BOP, (2) PPD ≥6mm without BOP and (3) PPD ≤3mm	Baseline
Heitz-Mayfield et al (2006)	Site-level treatment outcome defined as CAL gains of >3mm	Curette, subgingival biofilm sampled from the treated intrabony defect	Baseline (pre-surgery)
Keyes et al (2015)	Patient-level treatment outcome defined as the presence of 2 or more teeth exhibiting ≥3mm interproximal CAL, compared to baseline evaluations	Curette, pooled subgingival biofilm sampled from 2 to 5 periodontal sites with the greatest gingival inflammation, deepest residual probing depths, and/or furcation involvements	Post-APT
Kakuta et al (2017)	Patient-level periodontitis progression defined as at least 1 site exhibiting CAL ≥3mm during the 24-month study period	Paper points, subgingival biofilm sampled from the deepest pockets of each patient	Baseline

Detection method	Microbial biomarker outcome definition	Microbial biomarker	Odds ratio (95% CI)
Real-time PCR	Bacterial levels defined as the percentage of cells per total bacterial cell number present in subgingival biofilm sampled 3 months before progression	*P. gingivalis*	1.62 (1.16, 2.26)
		T. forsythia	2.30 (1.23, 4.32)
		T. denticola	0.74 (0.52, 1.05)[a]
Checkerboard DNA–DNA hybridisation	Positive test result defined by a high checkerboard score was defined as a score of 3 (>10^5 bacteria) or more, representing heavy colonisation	At least one "red complex[b]" species with a high score	39.33 (3.76–411.09)
		At least two "red complex" species with a high score	61.00 (5.46–681.58)
		At least one "complex A[c]" species with a high score	12.25 (1.28–117.72)
		At least two "complex A" species with a high score	28.50 (2.82–287.95)
		At least one "complex B[d]" species with a high score	14.57 (1.51–141.00)
		At least two "complex B" species with a high score	61.00 (2.42–134.12)
Checkerboard DNA–DNA hybridisation	Positive test result when *S. constellatus* comprises ≥3.5% of the total DNA probe count	*S. constellatus*	8.60 (NR)
Anaerobic culture	Positive test result defined by the presence of the target microbe	*A. actinomycetmcomitans*	1.68 (0.61–4.63)[a]
		P. gingivalis	0.49 (0.17–1.41)[a]
		P. intermedia	0.60 (0.09–4.16)[a]
		T. forsythia	0.80 (0.20–3.15)[a]
DNA-strip technology	Positive test result defined by the presence of the target microbe	*P. gingivalis* (3-month)	0.25 (0.06–1.01)[a]
Quantitative PCR	Positive test result when the percentage of *P. gingivalis*, *T. forsythia*, and *F. nucleatum*, exceeds 0.23%, 0.35% and 2.94% of the total bacteria, respectively	*P. gingivalis* (non-bleeding sites)	0.28 (0.10–0.70)
		P. gingivalis (bleeding sites)	0.68 (0.40–1.10)
		T. forsythia (non-bleeding sites)	0.53 (0.30–0.70)
		T. forsythia (bleeding sites)	0.55 (0.30–0.70)
		F. nucleatum (non-bleeding sites)	0.94 (0.50–1.60)[a]
		F. nucleatum (bleeding sites)	1.10 (0.80–1.50)[a]
Checkerboard DNA–DNA hybridisation	Test result defined as the bacterial counts (X 10^5) of individual species and complexes	Total counts	0.98 (0.96–0.99)
		Red complex counts[b]	0.99 (0.97–1.00)
		T. forsythia	0.98 (0.96–0.99)
		P. gingivalis	NR[a]
		T. denticola	NR[a]
Phase-contrast microscopy	Test results defined as elevated motile morphotype scores (100± or ≥ 125± per highest scoring microscopic fields (400x)	Elevated counts of medium- and large-size motile rods alone detected	3.90 (0.40–36.90)[a]
		Elevated counts of spirochetes alone detected	7.80 (1.70–35.70)
		Elevated counts of medium- and large-size motile rods and spirochetes detected concurrently	8.40 (1.90–38.30)
Invader PLUS assay, a PCR-based technique	Test results defined by each count (log_{10}) of each species	*P. gingivalis*	1.56 (1.03–2.34)
		P. intermedia	0.99 (0.52–1.76)[a]
		A. actinomycetemcomitans	44.70 (0.05–36.574)[a]

TABLE 3 (Continued)

Author (year)	Definition for treatment outcome	Sampling methodology	Timepoint
Mombelli et al (2017)	Patient-level treatment outcome defined as presence of at least 1 site with PPD >4 mm and BOP, 12 months post-therapy	Paper points, pooled subgingival biofilm from the deepest PPD from each quadrant	Baseline
Rams et al (1996)	Patient-level outcome defined as recurrent periodontitis at 6 and 12 months characterised by a PPD increase of ≥3 mm from baseline, or PPD increase ≥2 mm together with a CAL ≥2 mm from baseline	Paper points, pooled subgingival biofilm from deepest pocket of each sextant	Baseline

Abbreviations: CAL, clinical attachment loss; PCR, polymerase chain reaction; PPD, periodontal probing depth; BOP, bleeding on probing; NR, not reported.

[a]Denotes that the associations were not statistically significant.

[b]Red complex consists of *P. gingivalis*, *T. forsythia* and *T. denticola*.

[c]Complex A consists of *P. tannerae*, *F. alocis*, and *P. endodontalis*.

[d]Complex B consists of *P. intermedia*, *F. nucleatum* and *C. rectus*.

of *P. gingivalis* and *P. intermedia* in whole unstimulated saliva at the start of SPT were found to predict patients who would develop at least one site with additional CAL >3 mm during the next 18 months.[28] In another 24-month study, while a similar predictive value was observed for a high subgingival load of *P. gingivalis*, *P. intermedia* was not predictive of progressive periodontitis.[25] When scrutinising the predictive value of these biomarkers involving individual microbial species, Youden's index did not exceed 0.5, suggesting an overall low prognostic performance. In contrast, the highest predictive accuracy was achieved when at least 2 red complex species (*P. gingivalis*, *T. forsythia* and *T. denticola*) had presented with an elevated abundance (sensitivity of 0.80 and specificity of 0.91), improving Youden's index to 0.71.[19] However, it is important to exercise caution when interpreting the findings of this study due to its methodological limitations. Overall, while there was significant heterogeneity in the study designs and analytical approaches, a consistent finding was that an elevated abundance of periodontal pathogens at the start of SPT was predictive of subsequent periodontal disease progression.

4 | DISCUSSION

4.1 | Summary of key findings

The ideal periodontal prognostic biomarker should enable the non-invasive monitoring of the biological status at a periodontal site, providing accurate predictions of periodontal susceptibility, treatment response, and presence of progressive periodontitis. This systematic review evaluated the available evidence pertaining to the prognostic accuracy of microbial biomarkers and their associations with the response to active periodontal treatment and risks for progressive periodontitis during supportive periodontal therapy. Although the heterogenous methodology of the included studies precluded meta-analyses, this review provides several insights into the current status of clinical research on the prognostic value of periodontal microbial biomarkers.

While the investigated microbial species varied, significant impacts on treatment outcomes were observed when analysing the microbial load and abundance of specific periodontal pathogens but not with their presence or absence. These findings suggest that once a certain microbial load of pathogens is reached and maintained, the elicited inflammation exceeds the host threshold for inflammatory response, triggering chronic inflammation and tissue destruction. Here, these processes are expressed as non-resolving disease despite APT or progressive periodontitis during SPT. This concept of a critical microbial mass is no stranger to the periodontal community,[35] and is consistent with the classical studies[36,37] and our current understanding of the interactions between the dysbiotic biofilm and host response.[1] However, identifying universal thresholds for defining the critical microbial mass of periodontal pathogens would be challenging, as the disease processes of periodontitis are greatly influenced by host response and environmental factors.

Interestingly, although several studies evaluating the periodontal microbiome beyond specific species through sequence analysis were

Detection method	Microbial biomarker outcome definition	Microbial biomarker	Odds ratio (95% CI)
Quantitative PCR	Positive test defined as >1000 cells/mL at baseline	*A. actinomycetemcomitans*	1.42 (0.52–3.84)[a]
		P. gingivalis	0.92 (0.25–3.81)[a]
		P. intermedia	3.19 (1.02–12.22)
		T. forsythia	0.52 (0.02–13.54)[a]
		T. denticola	0.87 (0.20–4.55)[a]
		P. micra	3.79 (1.22–14.47)
		P. gingivalis, T. denticola and *T. forsythia* concomitantly	2.95 (1.05–9.22)
Anaerobic culture	Positive test result defined by the presence of ≥1 bacterial species (*A. actinomycetemcomitans, P. gingivalis, P. intermedia, C. rectus,* or *P. micros*)	≥1 bacterial species present (6 months)	3.01 (0.44–20.80)[a]
		≥1 bacterial species present (12 months)	1.20 (0.43–3.35)[a]
	Positive test result defined by ≥1 bacterial species recovered at or above the threshold proportions of ≥0.01% for *A. actinomycetemcomitans,* ≥0.1% for *P. gingivalis,* ≥2.5% for *P. intermedia,* ≥ 2.0% for *C. rectus,* ≥3.0% for *P. micros*	≥1 bacterial species recovered at or above the threshold proportions (6 months)	2.50 (0.77–8.14)[ay]
		≥1 bacterial species recovered at or above the threshold proportions (12 months)	2.50 (1.05–5.95)

included for full-text evaluation,[38-40] they were excluded from this review for not reporting quantitative measures of the association or prognostic accuracy of the biomarkers. While these next-generation sequencing studies provided much detail on the periodontal microbiome composition, highlighting key microbial differences in health, disease, and after successful treatment, this information was not translated into clinically applicable indices. A possible challenge hampering such translation is the difficulty in condensing the high dimensional data generated from sequence analysis into a clinically relevant composite microbial index. Recent advancements in machine learning techniques and artificial intelligence may contribute to this field. For example, Chen and co-workers have developed a subgingival microbial dysbiosis index (SMDI), through a machine learning analysis of prior published periodontal microbiome data.[41] This index is based on the species-level profiles associated with periodontitis, consisting of 19 discriminating genera which included several species that were also identified in this review. Further studies will be needed to assess the prognostic value of the SMDI for treatment response and validate its clinical utility.

While microbiome and metagenomic analyses provide insight into biofilm composition and potential for dysbiosis, individual microbial gene expression and the overall phenotype of the biofilm consortium are also greatly influenced by the microenvironment of the periodontal pocket and the host immune-inflammatory response. Microbial analyses are proximal biomarkers, and while of some value, may not be an accurate representation of biofilm functional and metabolic changes that are involved in the complex host-biofilm interactions. An alternative approach would be to directly evaluate the phenotype and virulence of the biofilm consortium. Since the periodontitis-associated biofilm predominantly consists of gram-negative anaerobes, analysing the immunogenicity of biofilm lipopolysaccharide, a highly conserved virulence factor, may provide insight into the biological status of the periodontal pocket. Indeed, a less favourable response to nonsurgical root debridement was reported in sites with longitudinal profiles of consistently high immunogenic LPS levels.[42] Notably, reports on these distal biomarkers are less common in the periodontal literature and would require additional studies to validate their utility.

4.2 | Limitations, clinical and research implications

This systematic review was limited by the inconsistent findings arising from the heterogenous methodology of the included studies. This was especially the case for active periodontal treatment, due to the wide range of treatment options available. This is further complicated by the difference in the biofilm sampled from untreated sites or following initial active treatment. Moreover, the associations and prognostic accuracy of the microbial biomarkers were assessed on different criteria as each study employed different definitions for "favourable treatment response." There is a need to address these sources of heterogeneity in future studies through the use of established evidence-based treatment endpoints, enabling meaningful comparisons of studies in the literature. Examples include "treat-to-target" defined as ≤4 sites with

PPD ≥5 mm, for the patient level,[43] and "pocket closure," characterised by PPD ≤4 mm without BOP, for the site level.[6] In addition, considering the chronic and dynamic nature of periodontitis, microbial biomarkers obtained from a single time point may provide limited insight, especially for long-term outcomes. Instead, these microbial biomarkers could be monitored longitudinally, identifying trajectory profiles that presage progressive periodontitis or a poor response to treatment, enabling effective interceptive treatment. Next-generation prognostic tools may be developed by integrating these microbial profiles with longitudinal clinical records and host-derived biomarkers, achieving a comprehensive surveillance of the disease processes of periodontitis.

5 | CONCLUSION

The development of effective periodontal prognostic tools is a critical hurdle limiting the implementation of a personalised medicine approach to periodontal therapy. Based on the findings of this systematic review, there is insufficient evidence to clinically implement microbial biomarkers as periodontal prognostic tools. However, biomarkers that quantify periodontal pathogen loads were found to be prognostic for progressive periodontitis during supportive periodontal therapy. Further research is required to translate this information regarding subgingival biofilm composition into clinically relevant prognostic tools.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in the supplementary materials of this study

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.