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Diagnostic Accuracy of a Point-Of-Care aMMP-8 Test for Discriminating Periodontal Health Status in Adults: Validation Trials and Updated Meta-Analysis

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ABSTRACT

Aim: To evaluate the diagnostic accuracy of an active matrix metalloproteinase-8 (aMMP-8) point-of-care oral rinse test (POC-ORT) for predicting periodontitis in treatment-naïve subjects in two independent studies and update a recent meta-analysis.

Methods: The aMMP-8 POC-ORT index test was performed in a representative population in Hong Kong, China, and a consecutive convenience sample in Shanghai, China. The reference standard was the 2017 World Workshop classification of periodontal diseases. Sensitivity, specificity, and the area under the receiver operating characteristic (AUROC) curve were assessed. The original data were used to update a recent Bayesian meta-analysis following the current Cochrane guideline for diagnostic trials. The GRADE framework was used to interpret the strength and certainty of the evidence.

Results: Three-hundred and eighty-four and 390 subjects were enrolled in the Hong Kong and Shanghai studies, respectively; 74.5% and 67.2% had periodontitis. An aMMP-8-positive test predicted periodontitis with an AUROC of 0.661 and 0.669 in the two studies. The updated systematic review and meta-analysis included eight studies and 2048 subjects. After considering the risk of bias, indirectness, inconsistency, imprecision and publication bias, it showed moderate certainty of a sensitivity of 0.59 (95% CrI: 0.42–0.75), a specificity of 0.82 (95% CrI: 0.68–0.93) and a hierarchical summary AUROC of 0.77 (95% CrI: 0.74–0.81).

Conclusion: There is moderate certainty that the aMMP-8 POC-ORT test predicts periodontitis with low to moderate sensitivity, moderate to high specificity, and moderate accuracy. Its high false-negative rate does not allow the replacement of clinical examinations when available. The moderate to high positive predictive value shows the potential utility of a positive test for self-detection or co-management of periodontitis in a medical setting and its incorporation in multi-test diagnostics. Further investigations are highly warranted.

Yuan Li and Julie Choi Ka Kung contributed equally to this research.

1 | Introduction

A biomarker is a characteristic that indicates normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention (FDA-NIH Biomarker Working Group 2016). Assays are analytical procedures for detecting a biomarker's presence, amount, state, or functional activity (Horvath et al. 2014). A valid assay of a biomarker may have diagnostic value if it can accurately predict a health outcome. Much research has focused on oral fluid biomarkers to discriminate periodontal health status (Bostanci and Belibasakis 2023). Discovery with proteomic approaches has identified differentially expressed markers in health, gingivitis and periodontitis (Ahmad et al. 2024; Hu and Leung 2023). Evidence from the diagnostic performance of single and multiple biomarkers has shown that translational research has focused on a relatively small subset of markers (Arias-Bujanda et al. 2020; Blanco-Pintos et al. 2023). These biomarkers align with the pathophysiology of periodontal disease and consist mainly of locally released inflammatory biomarkers or products of tissue destruction. Matrix metalloproteinase 8 (MMP8) is one of the most compelling candidates for discriminating periodontal health status (Arias-Bujanda et al. 2020; de Morais et al. 2018; Kc, Wang, and Gallagher 2020; Zhang et al. 2018).

After the discovery phase, biomarkers undergo studies to assess the analytical performance of a specific measuring assay. While early analytical performance studies are essential in the roadmap to developing a diagnostic test, they are rarely informative of diagnostic performance (Ferrante di Ruffano et al. 2012; Gürsoy and Kantarci 2022). Properly designed diagnostic trials comparing index and reference tests are necessary. A key element in their design is identifying, the setting or stage in the care pathway where the test will be employed. In this respect, a test can be used for screening, diagnosis, risk of progression or monitoring the treatment response. We have recently quantitatively summarized the diagnostic accuracy of trials assessing active-matrix metalloproteinase-8 (aMMP8), commercially available as a point-of-care oral rinse test (POC-ORT), for screening or diagnosis in treatment-naïve subjects (Wei et al. 2024). Using 20 ng/mL as a threshold for a positive test, the meta-analysis reported a mean sensitivity of 0.63 (95% confidence interval [CI]: 0.41–0.82), a mean specificity of 0.84 (95% CI: 0.65–0.95) for specificity and a mean area under the curve (AUC) of 0.82 (95% credible interval [CrI]: 0.62–0.95). Regarding accuracy, the values of the credible region of the summary receiver operating characteristic (SROC) curve intervals were too broad to draw conclusions about the applicability of aMMP8 as a screening or diagnostic test. The broad credible region may be due to study design heterogeneity, the small sample size of several studies, and the risk of bias due to test settings or participants. The overall assessment of the certainty of the evidence was downgraded from moderate to low because of concerns about the risk of bias in 67% of studies. More original research is needed to draw valid conclusions regarding the diagnostic accuracy of aMMP8 for the discrimination of periodontal health status.

The present study aimed to validate the diagnostic accuracy of aMMP8 for discriminating periodontitis at screening/initial diagnosis. We report two independent diagnostic trials with the same design and two sampling strategies. In Hong Kong, we recruited a random population sample, and in Shanghai we recruited a convenience sample of consecutive subjects seeking dental care. We then updated our recent meta-analysis and applied the Cochrane diagnostic trial grading tool to assess the certainty of the evidence for applying aMMP8 POCT for periodontitis.

2 | Materials and Methods

2.1 | Study Design and Populations

Two cross-sectional diagnostic accuracy studies assessing the performance of aMMP8 POC-ORT (index test) against full-mouth clinical periodontal examination and history conducted by periodontal specialists (reference test) were executed at the Prince Philip Dental Hospital in Hong Kong and the Ninth People's Hospital in Shanghai, China, between April 2022 and April 2024. The Hong Kong trial population was recruited from a representative list of household addresses obtained by the systematic random sampling method generated from the addresses of households provided by the Census and Statistics Department of the Hong Kong Special Administrative Region, as previously reported (Department of Health, Hong Kong SAR Government 2011). Subjects were invited to participate in the study by replying to a letter mailed to their residential address. The Shanghai trial comprised a convenience sample of consecutive subjects seeking dental care at the hospital. All subjects aged 18 or above were invited to participate. The exclusion criteria were (1) edentulous adults, (2) pregnant females, (3) subjects who received antibiotics within the previous 3 months, and (4) subjects who received professional periodontal treatment (other than supragingival cleaning) within the previous 12 months. The study protocols were approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB Approval Number: UW22-132; trial registration NCT03928080) and of the Shanghai Ninth People's Hospital Ethics Committee (IRB Approval Number: SH9H-2021-T408-3; trial registration: NCT05513599). All procedures were conducted following the current revision of the Declaration of Helsinki. All participants provided written consent before the start of the study. This study followed the Standards for Reporting Diagnostic Accuracy (STARD) guidelines (Cohen et al. 2016).

2.2 | Sample Size Estimation

The sample size was estimated based on Deng et al. (2022) using the confidence interval method and the following assumptions: an actual area under the receiver operating characteristic (AUROC) curve of 0.7 and a 2:1 positive to negative test result ratio. Calculations were performed with Pass 15.0.5 (NCSS LLC, Utah, USA). A sample of 380 subjects will provide a 95% CI to detect a width of 0.1 between the upper and lower confidence limits of the AUROC.

2.3 | Oral Rinse aMMP-8 Point-Of-Care Index Test

The index test was performed after sample collection with a commercial aMMP-8 POC-ORT system (PerioSafe PRO,

Dentognostics GmbH, Jena, Germany) and its digital analysis device (ORALyzer, Dentognostics GmbH, Jena, Germany) as previously shown by our team (Deng et al. 2021, 2022). In brief, the test is a lateral flow immunoassay that quantifies aMMP-8 at concentrations with a reported detection level of 10 ng/mL. It is based on Sorsa et al.'s invention (U.S. Patent No. 10488415, 2019). Following the manufacturer's instructions (http://perioprevention center.com/professionals/products/oralyzer-ammp-8-digital-reader/), the test was performed using 3–4 drops of filtered 30-s oral rinse with a proprietary buffer, as described Deng et al. (2022). Following the manufacturer's recommendation, the diagnostic performance of the aMMP-8 test results was assessed using the cut-off value of 20 ng/mL. Quantitative results of the aMMP-8 test were also related to the number of teeth present by dividing the total concentration by the number of teeth present (aMMP-8/NTP). For this latter analysis, aMMP-8 levels below the detection level were considered 10 ng/mL for the statistical analysis.

2.4 | Periodontal Examination and Case Definition: Reference Standard

As performed and interpreted by the calibrated examiners (XW, SL and JK), full-mouth periodontal examinations were the reference standard for diagnosing periodontal health, gingivitis and different stages and grades of periodontitis (Deng et al. 2021). The clinical assessment involved using a periodontal probe (UNC-15, Hu Friedy, Chicago) to measure the probing pocket depth (PPD), full-mouth bleeding score (FMBS) and clinical attachment level (CAL) at six sites per tooth. Additionally, the examination included assessing furcation involvement (FI) and tooth mobility and determining the number of teeth lost due to periodontitis. Before the study, all examiners were trained and calibrated for PPD and CAL measurements and case diagnosis and achieved a kappa value within 1 mm of >0.85. Duplicate measurements were performed on every 30 subjects, and kappa values remained better than 0.85 throughout the study. Self-reported demographic characteristics, smoking status and medical history with particular emphasis on diabetes and its control were also collected.

The 2017 classification of periodontal diseases was used to diagnose various periodontal conditions, such as periodontal health, gingivitis, and periodontitis stages and grades. Unaware of the aMMP-8 test results, the examiners employed the algorithm suggested by Tonetti and Sanz (2019) to diagnose each case. This study used periodontal disease to denote subjects with plaque-induced gingivitis or periodontitis. The grade of periodontitis was determined by evaluating radiographic bone loss in relation to age on digital orthopantomographic images and assessing periodontal destruction based on plaque and calculus deposits. The grade was adjusted depending on self-reported smoking and diabetes control (Tonetti and Sanz 2019). The extent of periodontal disease was assessed by measuring the percentage of bleeding during probing for cases of gingivitis (Chapple et al. 2018). Additionally, a 30% threshold of affected teeth at the most severe stage was employed to distinguish between localized and generalized periodontitis (Sanz et al. 2020). The investigators discussed subjects with unclear status to reach a consensus.

2.5 | Data Analysis and Meta-Analysis

Mean values and standard deviations (SDs) are used to present continuous variables, while frequency distributions are used for categorical variables. Differences in continuous variables among patient groups were tested using the non-parametric Kruskal–Wallis test. Comparisons of differences in categorical variables were conducted using the chi-square test and Fisher's exact test. To evaluate the effectiveness of the binary aMMP-8 POC-ORT in distinguishing between different periodontal case definitions, sensitivity, specificity, positive predictive value (PPV), as well as and negative predictive value (NPV) were calculated using a chi-square test with a threshold of 20 ng/mL. The diagnostic accuracy and odds ratios (DOR) of the aMMP-8 test results were assessed using logistic regression to predict periodontal health status. Model 1 was a crude analysis of a positive aMMP-8 test. Model 2 was a crude analysis of aMMP-8/NTP. Model 3 was the selection of the best significant subset of variables using risk factors/indicators and models 1 and 2. ROC curves were created for each model, and various thresholds for aMMP-8/NTP or predicted probability levels were used to estimate the area under the ROC curve (AUROC), as well as sensitivity and specificity. For aMMP-8/NTP, the corresponding cut-off value was determined by optimizing sensitivity and specificity from the ROC curves. Sensitivity and specificity values were defined to be low ($<60\%$), moderate (60%–79%) or high (80%) (Nelson et al. 2001). The diagnostic accuracy results derived from the AUROC values were interpreted as low level (0.50–0.70), moderate level (0.71–0.90) and high level (>0.90) (Swets 1988). p-Values <0.05 were considered to be statistically significant. Statistical analyses were performed using SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). Results were used to update a recently published meta-analysis using the same methodology reported in the original publication (Wei et al. 2024). In brief, quantitative analyses were performed using Stata (v18.1; StataCorp, College Station, TX, USA), R (v4.3.1; The R Foundation for Statistical Computing Platform), and MetaBayesDTA (Cerullo et al. 2023). The diagnostic measures of aMMP-8 POC-ORT for detecting periodontitis were evaluated across different studies following the relevant Cochrane guidelines for evaluating medical tests (Bossuyt et al. 2023). Before entering the present studies in the meta-analysis, their risk of bias was assessed with the QUADAS-2 tool. The strength and certainty of the evidence were evaluated using the GRADE framework to interpret the results.

3 | Results

3.1 | Experimental Populations

Two thousand letters were mailed twice to invite participants for the Hong Kong study. From 411 responses (20.6% response rate), 384 subjects were successfully recruited (19.2% participation rate). The Shanghai sample comprised 390 consecutive participants drawn from 443 potentially eligible subjects (88% consent rate). Table 1 shows their demographics, risk factor profiles, and periodontal parameters by reference case definition. Two-hundred and sixty-two (67.2%) and 286 (74.5%) had periodontitis in Shanghai and Hong Kong, respectively. Age, sex, and clinical parameters significantly

TABLE 1 | Demographics and periodontal parameters of the two study populations.

Hong Kong study		Total ($n=384$)	Perio health ($n=24$)	Gingivitis ($n=74$)	Periodontitis ($n=286$)	Stage I ($n=12$)	Stage II ($n=62$)	Stage III ($n=169$)	Stage IV ($n=43$)	p
Demographics										
Gender	Male	146 (38.0%)	7 (29.2%)	29 (39.2%)	110 (38.5%)	4 (33.3%)	14 (22.6%)	73 (43.2%)	19 (44.2%)	0.08
Age, years		55.3 ± 16.6	28.8 ± 7.3	39.5 ± 14.9	61.7 ± 12.1	36.3 ± 11.7	58.9 ± 12.0	62.8 ± 10.3	68.7 ± 18.1	< 0.001
	18–29	45 (11.7%)	18 (75.0%)	23 (31.1%)	4 (1.4%)	3 (25.0%)	1 (1.6%)	0	0	< 0.001
	30–59	137 (35.7%)	6 (25.0%)	40 (54.0%)	91 (31.8%)	9 (75.0%)	28 (37.1%)	55 (32.5%)	4 (9.3%)	
	≥60	202 (52.6%)	0	11 (14.9%)	191 (66.8%)	0	38 (61.3%)	114 (67.5%)	39 (90.7%)	
Smoking status	Non-smokers	342 (89.1%)	23 (95.8%)	65 (87.8%)	254 (88.8%)	12 (100%)	56 (90.3%)	150 (88.8%)	36 (83.7%)	0.453
	Current	17 (4.4%)	0	3 (4.1%)	14 (4.9%)	0	0	10 (5.9%)	4 (9.3%)	0.453
	Former	25 (6.5%)	1 (4.2%)	6 (8.1%)	18 (6.3%)	0	6 (9.7%)	9 (5.3%)	3 (7.0%)	0.453
Systemic disease	Healthy	242 (63.0%)	23 (95.8%)	65 (87.8%)	214 (76.4%)	10 (83.3%)	44 (71.0%)	83 (49.1%)	17 (39.5%)	< 0.001
	CVD	115 (30%)	0	9 (12.2%)	44 (15.7%)	2 (16.7%)	12 (19.4%)	72 (42.6%)	20 (46.5%)	< 0.001
	DM	41 (10.7%)	0	2 (2.7%)	20 (7.1%)	0	4 (6.5%)	24 (14.2%)	11 (25.6%)	< 0.001
	PC-DM	12 (3.1%)	0	0	12 (4.3%)	0	1 (1.6%)	8 (4.7%)	3 (7.0%)	0.202
Clinical parameters										
Number of teeth		26.4 ± 4.4	27.8 ± 1.5	28.6 ± 2.0	25.7 ± 4.6	28.5 ± 2.2	27.7 ± 2.2	26.5 ± 3.3	19.1 ± 6.1	< 0.001
FMBS		50.3 ± 27.1	7.2 ± 2.7	37.7 ± 20.8	57.1 ± 25.1	52.4 ± 25.2	42.8 ± 22.4	58.7 ± 24.1	72.8 ± 21.9	< 0.001
PPD (mm)		2.58 ± 0.67	2.02 ± 0.12	2.17 ± 0.27	2.74 ± 0.70	2.30 ± 0.39	2.67 ± 0.31	2.80 ± 0.66	3.30 ± 0.80	< 0.001
CAL (mm)		2.71 ± 1.81	0.62 ± 0.52	0.67 ± 0.29	3.41 ± 1.55	1.86 ± 0.68	1.88 ± 0.73	3.58 ± 1.14	5.39 ± 1.38	< 0.001
FI ≥ II (%)		3.6 ± 6.7	0	0	4.83 ± 7.37	0	0	5.77 ± 7.06	9.46 ± 10.13	< 0.001
Mobility ≥ II (%)		1.3 ± 4.2	0	0	1.73 ± 4.84	0	0	1.02 ± 2.38	7.47 ± 9.77	< 0.001
Periodontitis grades										
Grade A		N/A	N/A	N/A	22 (7.7%)	9 (75.0%)	13 (21.0%)	0	0	< 0.001
Grade B		N/A	N/A	N/A	143 (50.0%)	3 (25.0%)	48 (77.4%)	86 (50.9%)	6 (14.0%)	< 0.001
Grade C		N/A	N/A	N/A	121 (42.3%)	0	1 (1.6%)	83 (49.1%)	37 (86.0%)	< 0.001

(Continues)

TABLE 1 | (Continued)

Hong Kong study		Total ($n=384$)	Perio health ($n=24$)	Gingivitis ($n=74$)	Periodontitis ($n=286$)	Stage I ($n=12$)	Stage II ($n=62$)	Stage III ($n=169$)	Stage IV ($n=43$)	p
Periodontal disease extent										
Localized		N/A	N/A	30 (40.5%)	115 (40.2%)	9 (75.0%)	23 (37.1%)	83 (49.1%)	0	<0.001
Generalized		N/A	N/A	44 (59.5%)	171 (59.8%)	3 (25.0%)	39 (62.9%)	86 (50.9%)	43 (100%)	<0.001
Shanghai study		**Total ($n=390$)**	**Perio health ($n=10$)**	**Gingivitis ($n=118$)**	**Periodontitis ($n=262$)**	**Stage I ($n=153$)**	**Stage II ($n=28$)**	**Stage III ($n=64$)**	**Stage IV ($n=17$)**	**p**
Demographics										
Gender	Male	132 (33.9%)	3 (30%)	23 (19%)	106 (59.5%)	47 (30.7%)	12 (42.9%)	36 (56.3%)	11 (64.7%)	<0.001
	Female	258 (66.1%)	7 (70%)	95 (81%)	156 (40.5%)	106 (69.3%)	16 (57.1%)	28 (43.7%)	6 (35.3%)	
Age, years		30 ± 8	38 ± 12	23 ± 4	33 ± 13	25 ± 6	33 ± 13	45 ± 12	54 ± 10	<0.001
	18–29	261 (66.9%)	4 (40%)	114 (96.6%)	143 (54.6%)	124 (81.1%)	15 (53.6%)	4 (6.3%)	0	<0.001
	30–59	115 (29.5%)	5 (50%)	4 (3.4%)	106 (40.5%)	29 (18.9%)	12 (42.9%)	53 (82.8%)	12 (70.6%)	
	≥60	14 (3.6%)	1 (10%)	0	13 (4.9%)	0	1 (3.5%)	7 (10.9%)	5 (29.4%)	
Smoking status	Non-smokers	349 (89.5%)	8 (80%)	115 (97.5%)	226 (86.3%)	143 (93.5%)	24 (85.7%)	47 (73.4%)	12 (70.6%)	<0.001
	Former	17 (4.4%)	1 (10%)	1 (0.8%)	15 (5.7%)	3 (1.9%)	1 (3.6%)	8 (12.5%)	3 (17.6%)	
	Current	24 (6.1%)	1 (10%)	2 (1.7%)	21 (8%)	7 (4.6%)	3 (10.7%)	9 (14.1%)	2 (11.8%)	
Systemic conditions	Healthy	269 (69%)	9 (90%)	96 (82.1%)	164 (62.6%)	107 (69.9%)	16 (57.1%)	33 (51.6%)	8 (47.1%)	<0.001
	CVD	15 (3.8%)	0	2 (0.8%)	13 (5%)	5 (3.3%)	1 (3.6%)	6 (9.4%)	1 (5.8%)	0.186
	DM	2 (0.5%)	0	0	2 (0.7%)	0	0	2 (3.1%)	0	
	PC-DM	0	0	0	0	0	0	0	0	
	Others	104 (26.7%)	1 (10%)	20 (17.1%)	83 (31.7%)	41 (26.8%)	11 (39.3%)	23 (35.9%)	8 (47.1%)	<0.001
Clinical parameters										
Number of teeth		26.7 ± 1.8	26.2 ± 1.93	27.5 ± 1.10	26.4 ± 2.37	27 ± 1.39	26.3 ± 1.76	25.98 ± 1.44	22 ± 5.92	<0.0001
FMBS		59.38 ± 15.68	8.77 ± 1.15	52.71 ± 15.47	64.31 ± 16.68	60.49 ± 15.47	72.86 ± 15.70	67.27 ± 16.61	73.46 ± 19.32	<0.0001
PPD (mm)		3.09 ± 0.45	2.27 ± 0.12	2.91 ± 0.23	3.21 ± 0.6	3.06 ± 0.28	3.16 ± 0.41	3.30 ± 0.54	4.24 ± 1.53	<0.0001
CAL (mm)		0.77 ± 0.71	0.25 ± 0.18	0.04 ± 0.05	1.11 ± 1.67	0.18 ± 0.16	0.69 ± 0.33	2.44 ± 1.25	5.23 ± 2.3	<0.0001

(Continues)

TABLE 1 | (Continued)

Shanghai study	Total ($n = 390$)	Perio health ($n = 10$)	Gingivitis ($n = 118$)	Periodontitis ($n = 262$)	Stage I ($n = 153$)	Stage II ($n = 28$)	Stage III ($n = 64$)	Stage IV ($n = 17$)	p
FI ≥ II (%)	2.05 ± 3.13	0	0	3.04 ± 6.29	0	0	7.91 ± 6.78	17.13 ± 7.41	< 0.0001
Mobility ≥ II (%)	0.88 ± 5.47	0	0	1.31 ± 7.91	0	0	0.71 ± 1.76	17.58 ± 26.56	< 0.0001
Periodontitis grades									
Grade A	N/A	N/A	N/A	29 (11.1%)	29 (19%)	0	0	0	< 0.0001
Grade B	N/A	N/A	N/A	181 (69.1%)	124 (81%)	23 (82.1%)	32 (50%)	2 (11.8%)	< 0.0001
Grade C	N/A	N/A	N/A	52 (19.8%)	0	5 (17.9%)	32 (50%)	15 (88.2%)	< 0.0001
Periodontal disease extent									
Localized	N/A	N/A	12 (10.2%)	166 (63.4%)	100 (65.4%)	27 (96.4%)	38 (59.4%)	1 (5.9%)	< 0.0001
Generalized	N/A	N/A	106 (89.8%)	96 (36.6%)	53 (34.6%)	1 (3.6%)	26 (40.6%)	16 (94.1%)	

Note: Data were presented as either mean ± SD or *n* (%); Stage I to IV refers to the different Stages of Periodontitis. FMBS: full mouth bleeding score; PPD: probing pocket depth; CAL: clinical attachment loss; FI: furcation involvement; N.A.: not applicable; Grade definitions were only applicable to Periodontitis subjects; Extent assessment was only applied to Gingivitis and Periodontitis. H, periodontal health; G, gingivitis; I, Stage I periodontitis; II, Stage II periodontitis; III, Stage III periodontitis; IV, Stage IV periodontitis; P, periodontitis. CVD, cardiovascular diseases; DM, diabetes mellitus; PC-DM: Poorly controlled diabetes mellitus: HbA1c ≥ 7.0%; Chi-square tests or Fisher's exact tests (for categorical data) and Kruskal-Wallis tests (for continuous data) were used to assess differences among groups.

differed across case diagnoses in both studies. Self-reported smoking was significantly associated with poorer periodontal health status in Shanghai but not in Hong Kong. The reverse was true for type II diabetes. Only a few subjects self-reported smoking or diabetes. The STARD diagrams are shown in Figure S1.

3.2 | Accuracy of a Positive aMMP-8 POC-ORT to Discriminate Periodontal Case Definitions and Periodontitis Grade

The discriminative utility of the dichotomized aMMP-8 POC-ORT (threshold 20 ng/mL) to identify different periodontal case definitions is presented in Table 2. Positive tests were more frequently observed in periodontitis subjects in both populations ($p < 0.001$). In addition, subjects with more advanced periodontitis stages showed significantly higher rates of positive tests ($p = 0.023$ in Hong Kong and $p < 0.001$ in Shanghai). No differences were observed when periodontal health was compared with gingivitis. Table 3 shows the accuracy of aMMP8 POC-ORT in predicting periodontitis extent and grade. The rate of true positives was significantly higher in grade C cases compared to grade A in both studies. No consistency across the two studies was observed for the prediction of extent.

3.3 | Association of the aMMP-8 Test Results With Periodontitis Case Definition, Periodontitis Grade and Periodontal Clinical Parameters

Logistic regression analysis examined associations between aMMP-8 POC-ORT results and periodontitis (Table 4a,b). Statistically significant correlations were found between positive aMMP-8 POC-ORT results or aMMP-8/NTP levels and total periodontitis, including stages I/II, III, and IV. However, the Hong Kong study showed no significant link between stage IV periodontitis and positive aMMP-8 results. Furthermore, the Shanghai study lacked sufficient healthy subjects for comparison. After adjusting for age, gender, smoking, and systemic disease, associations remained significant. Table 4c presents the correlation between aMMP-8 POC-ORT results and periodontal parameters. In both populations, subjects with positive POC-ORT results had higher odds ratios for periodontal pockets (PPD ≥ 4 mm), deep pockets (PPD ≥ 6 mm), and bleeding pockets compared to negatives. Furthermore, aMMP-8/NTP levels positively correlated with the number of periodontal pockets. However, valid comparisons for PPD ≥ 4 mm in the Shanghai study were not possible because of a limited number of subjects without pockets or bleeding. Table 4d illustrates the correlation between aMMP-8 POC-ORT levels and the grade of periodontitis. In both studies, subjects classified with grade C periodontitis demonstrated a greater number of positive aMMP-8 results or elevated levels of aMMP-8/NTP compared to those in grades A and B.

3.4 | Effect of Demographics and Risk Factors

Logistic regression models were constructed to model the accuracy of aMMP8 (Model 1) or aMMP8/NTP (Model 2) alone or after the incorporation of demographics and risk factors (Model 3) for the prediction of periodontitis stage. Results are shown

TABLE 2 | Accuracy of the dichotomized aMMP-8 POC-ORT (threshold 20 ng/mL) to discriminate various periodontal case definitions.

Diagnostic accuracy measures	Periodontal case definitions									
	Periodontal disease	Gingivitis	Periodontitis	The stages of periodontitis				*p*	*p*	*p*
				I	II	III	IV	P(I–IV)/NP	G/H	I–IV
Hong Kong study	*N* = 360	*N* = 74	*N* = 286	*N* = 12	*N* = 62	*N* = 169	*N* = 43			
Test positive (*n*/%)	150 (41.7%)	14 (18.9%)	136 (47.6%)	4 (33.3%)	20 (32.3%)	90 (53.3%)	22 (51.2%)	< 0.001	0.081	0.023
Test negative (*n*/%)	210 (58.3%)	60 (81.1%)	150 (52.4%)	8 (66.7%)	42 (67.7%)	79 (46.7%)	21 (48.8%)			
Performance										
Sensitivity	41.7%	18.9%	47.6%	33.3%	32.3%	53.3%	51.2%			
Specificity	95.8%	55.8%	84.7%	60.5%	59.3%	71.6%	62.1%			
Positive predictive value	99.3%	9.3%	90.1%	2.7%	13.3%	59.6%	14.6%			
Negative predictive value	9.9%	74.3%	35.6%	96.6%	82.0%	66.1%	91.0%			
Shanghai study	*N* = 380	*N* = 118	*N* = 262	*N* = 153	*N* = 28	*N* = 64	*N* = 17			
Test positive (*n*/%)	153 (40.3%)	21 (17.8%)	132 (50.4%)	64 (41.8%)	13 (46.4%)	41 (64.1%)	14 (82.4%)	< 0.001	0.1445	< 0.001
Test negative (*n*/%)	227 (59.7%)	97 (82.2%)	130 (49.6%)	89 (58.2%)	15 (53.6%)	23 (35.9%)	3 (17.6%)			
Performance										
Sensitivity	40.3%	17.8%	50.4%	41.8%	46.4%	64.1%	82.4%			
Specificity	100%	48.5%	83.6%	62.4%	61.3%	65.6%	62.7%			
Positive predictive value	100%	40.9%	86.3%	41.8%	8.5%	26.8%	9.2%			
Negative predictive value	4.2%	86.3%	45.1%	62.4%	93.7%	90.3%	98.7%			

Note: Periodontal disease = gingivitis + periodontitis. Chi-square tests were used to assess differences between groups. Performance refers to discriminating periodontal disease or gingivitis from periodontal health and discriminating periodontitis (stages I–IV) from non-periodontitis. H, periodontal health; G, gingivitis; I, stage I periodontitis; II, stage II periodontitis; III, stage III periodontitis; IV, stage IV periodontitis; P, periodontitis; NP, non-periodontitis.

TABLE 3 | Utility of the dichotomized aMMP-8 POC-ORT (threshold 20 ng/mL) to discriminate various grades and extent of periodontitis.

Diagnostic accuracy measures	Periodontitis						
	Extent		Grade			*p*	*p*
	Localized	Generalized	Grade A	Grade B	Grade C	Loc/Gen	A–C
Hong Kong study	*N* = 115	*N* = 171	*N* = 22	*N* = 143	*N* = 121		
Test positive (*n*/%)	45 (39.1%)	91 (53.2%)	8 (36.4%)	49 (34.3%)	79 (65.3%)	0.019	< 0.001
Test negative (*n*/%)	70 (60.9%)	80 (46.8%)	14 (63.6%)	94 (65.7%)	42 (34.7%)		
Performance							
Sensitivity	39.1%	53.2%	36.4%	34.3%	63.3%		
Specificity	46.8%	60.1%	51.5%	39.2%	65.5%		
Positive predictive value	33.1%	66.9%	5.9%	36.0%	58.1%		
Negative predictive value	55.3%	46.7%	90.7%	37.3%	72.0%		
Shanghai study	*N* = 166	*N* = 96	*N* = 29	*N* = 181	*N* = 52		
Test positive (*n*/%)	78 (47%)	54 (56.3%)	3 (10.3%)	96 (53%)	33 (63.5%)	0.149	< 0.001
Test negative (*n*/%)	88 (53%)	42 (43.7%)	26 (89.7%)	85 (47%)	19 (36.5%)		
Performance							
Sensitivity	47%	56.3%	10.3%	53%	63.5%		
Specificity	56.3%	53%	55.4%	55.6%	52.9%		
Positive predictive value	67.7%	40.9%	20%	72.7%	25%		
Negative predictive value	40.9%	67.7%	97.7%	34.6%	85.4%		

Note: Chi-square tests were used to assess differences between groups. Performance refers to distinguishing localized (loc) from generalized (gen) periodontitis and grade A periodontitis from grade C.

in Table 5. AUROC values were generally consistent in the two studies. For periodontitis, models with aMMP8 showed AUROC values of 0.661 in Hong Kong and 0.669 in Shanghai. Models incorporating the number of teeth present (Model 2) showed better accuracy, with an AUROC of 0.768 in Hong Kong and 0.769 in Shanghai. The inclusion of demographics (Model 3) further improved the accuracy. As expected, models performed better in predicting more advanced stages of periodontitis.

The same models were tested to predict periodontitis grade and extent (Table 6). For grade, the AUROC of Models 1 and 2 were similar to those observed for the periodontitis stage. Because of the definition of grade, age, sex, and smoking in Model 3 were significant and improved the AUROC. Age was the only substantial factor observed in the two studies regarding the extent.

3.5 | Updated Meta-Analysis

An update of a recent meta-analysis was performed to enable interpretation of the present data as part of the whole body of evidence. The QUADAS-2 quality assessment showed that the present two studies were at low risk of bias (Figure S2). The body of included evidence comprised eight studies with 2048 subjects. Figure 1 shows the results of the updated meta-analysis. A pooled sensitivity of 0.59 (95% CI: 0.42–0.75) and a specificity of 0.82 (95% CI: 0.68–0.93) were observed (Figure 1A). Figure 1B shows the summary ROC (SROC) plot. The bubbles in the plot represent the included studies, and their size shows the sample size. Adding the present data enabled increased precision on the credible region (area delimited by the dashed blue line). Figure 1C shows the plot of each study's contribution to overall heterogeneity and influence on the overall result. The random effect estimate of the DOR was 4.62 (95% CI: 3.45–6.18). DORs were stable, leaving one study out (Figure 1D), and the Deeks funnel plot shows no evidence of publication bias (Figure 1E). The strength of the evidence was judged moderate because of the risk of bias in some studies and between-study variability. A sensitivity analysis was performed, including only studies at low risk of bias. It showed decreased summary sensitivity and stable summary specificity (Figure S3).

The certainty of the evidence was evaluated using the five domains of risk of bias, indirectness, inconsistency, imprecision, and publication bias (Table 7). Based on the wide confidence intervals of the summary estimates, it was downgraded by one level to moderate. The rationale was that lower and upper limits of the credible intervals would lead to different decisions regarding adopting the test.

TABLE 4 | Logistic regression analyses for the association of the aMMP-8 test results with periodontitis case definition and periodontal clinical parameters (the threshold value for the aMMP-8 test is set at 20 ng/mL).

(a) Periodontitis

	Periodontitis versus periodontal health		Periodontitis versus gingivitis		Periodontitis versus periodontal health + gingivitis	
Variables	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Hong Kong study						
Male	1.53 (0.61–3.8)		0.98 (0.58–1.65)		1.08 (0.67–1.74)	
Age	1.24 (1.14–1.34)***	1.24 (1.13–1.35)***	1.11 (1.09–1.13)***	1.11 (1.09–1.14)***	1.12 (1.10–1.15)***	1.12 (1.10–1.15)***
Current smokers	/	/	1.22 (0.34–56.73)		1.63 (0.46–5.80)	
Systemic disease	5.60 (0.74–42.36)**		5.76 (1.75–18.97)**	/	5.72 (2.02–16.23)**	
aMMP-8 positive test	20.85 (2.78–156.50)**		3.89 (2.08–7.29)***	4.11 (1.92–8.81)***	5.02 (2.76–9.11)***	4.42 (2.09–9.35)***
aMMP-8/NTP	41.91 (2.30–764.71)*	28.26 (1.73–461.86)*	2.32 (1.51–3.56)***		2.81 (1.80–4.39)***	
Shanghai study						
Male	1.59 (0.40–6.3)		2.81 (1.67–4.71)***	1.97 (1.09–3.58)*	2.67 (1.62–4.38)***	1.89 (1.07–3.31)*
Age	0.97 (0.93–1.02)		1.18 (1.12–1.25)***	1.17 (1.10–1.24)***	1.11 (1.07–1.15)***	1.08 (1.05–1.13)***
Current smokers	0.78 (0.10–6.49)		5.05 (1.17–21.92)*		3.63 (1.06–12.40)*	
Systemic disease	5.38 (0.67–43.09)		2.61 (1.54–4.41)***		2.73 (1.63–4.57)***	1.95 (1.10–3.48)*
aMMP-8 positive test	Insufficient observations for valid comparisons		4.69 (2.76–7.97)***		5.17 (3.06–8.76)***	
aMMP-8/NTP			5.06 (2.82–9.08)***	4.43 (2.39–8.22)***	5.59 (3.10–10.09)***	5.07 (2.74–9.37)***

(b) Different stages of periodontitis

	Stages I/II periodontitis versus periodontal health + gingivitis		Stage III periodontitis versus periodontal health + gingivitis + stages I/II periodontitis		Stage IV periodontitis versus periodontal health + gingivitis + stages I–III periodontitis	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Hong Kong study						
Male	0.56 (0.29–1.10)		0.75 (0.48–1.16)	2.05 (1.14–3.70)*	1.33 (0.70–2.52)	
Age	1.08 (1.06–1.11)***	1.08 (1.06–1.11)***	1.09 (1.07–1.11)***	1.10 (1.07–1.12)***	1.09 (1.06–1.13)***	1.09 (1.05–1.14)***
Current smokers	/		3.54 (0.96–13.11)	5.09 (1.14–22.84)*	2.59 (0.80–8.33)	5.92 (1.55–22.66)**

(Continues)

TABLE 4 | (Continued)

(b) Different stages of periodontitis

	Stages I/II periodontitis versus periodontal health + gingivitis		Stage III periodontitis versus periodontal health + gingivitis + stages I/II periodontitis		Stage IV periodontitis versus periodontal health + gingivitis + stages I–III periodontitis	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Systemic disease	3.67 (1.10–12.22)*		2.84 (1.46–5.52)**		2.36 (1.14–4.92)*	
aMMP-8 positive test	2.66 (1.27–5.54)**	2.58 (1.09–6.10)*	3.89 (2.43–6.20)***	3.33 (1.90–5.82)***	1.27 (0.67–2.42)	
aMMP-8/NTP	1.56 (1.04–2.33)*		1.85 (1.44–2.38)***		1.42 (1.22–1.65)***	1.32 (1.13–1.54)***
Shanghai study						
Male	2.15 (1.28–3.61)**	1.79 (1.01–3.16)*	3.39 (1.95–5.89)***		3.82 (1.38–10.57)**	
Age	1.07 (1.04–1.11)***	1.06 (1.02–1.10)**	1.18 (1.14–1.22)***	1.18 (1.14–1.23)***	1.13 (1.08–1.17)***	1.12 (1.07–1.17)***
Current smokers	2.69 (0.74–9.72)		3.73 (1.52–9.14)**		2.13 (0.46–9.89)	
Systemic disease	2.34 (1.36–4.00)**	1.94 (1.08–3.50)*	2.64 (1.52–4.59)***		2.62 (0.99–6.97)	
aMMP-8 positive test	4.33 (2.51–7.47)***		3.84 (2.18–6.75)***	5.78 (2.52–13.22)***	7.86 (2.22–27.82)**	
aMMP-8/NTP	4.70 (2.58–8.56)***	4.75 (2.56–8.82)***	2.66 (1.89–3.74)***		2.76 (1.92–3.96)***	2.07 (1.39–3.09)***

(c) Periodontal clinical parameters

	≥ 2 sites with PPD ≥ 4 mm versus no sites with PPD ≥ 4 mm and 1 site with PPD = 4 mm		≥ 1 site with bleeding pocket (PPD ≥ 4 mm) versus no sites with bleeding pocket (PPD ≥ 4 mm)		≥ 1 site with PPD ≥ 6 mm versus no sites with PPD ≥ 6 mm		≥ 1 site with bleeding pocket (PPD ≥ 6 mm) versus no sites with bleeding pocket (PPD ≥ 6 mm)	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Hong Kong study								
Male	1.78 (1.07–2.97)*		1.88 (1.06–3.33)*		1.53 (1.01–2.31)*		1.54 (1.01–2.33)*	
Age	1.04 (1.02–1.05)***	1.03 (1.01–1.04)**	1.04 (1.02–1.05)***	1.03 (1.01–1.04)**	1.03 (1.02–1.05)***	1.03 (1.01–1.04)***	1.03 (1.02–1.05)***	1.03 (1.01–1.04)***
Current smokers	5.28 (0.69–40.33)		3.77 (0.49–28.92)		3.11 (1.08–8.99)*	3.76 (1.22–11.55)*	3.21 (1.11–9.30)*	3.98 (1.29–12.28)*
Systemic disease	1.95 (0.92–4.14)		1.34 (0.63–2.87)		1.39 (0.80–2.41)		1.44 (0.83–2.50)	

(Continues)

TABLE 4 | (Continued)

(c) Periodontal clinical parameters

	≥2 sites with PPD ≥4 mm versus no sites with PPD ≥4 mm and 1 site with PPD = 4 mm		≥1 site with bleeding pocket (PPD ≥4 mm) versus no sites with bleeding pocket (PPD ≥4 mm)		≥1 site with PPD ≥6 mm versus no sites with PPD ≥6 mm		≥1 site with bleeding pocket (PPD ≥6 mm) versus no sites with bleeding pocket (PPD ≥6 mm)	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
aMMP-8 positive test	6.06 (3.17–11.59)***		7.79 (3.46–15.54)***		5.37 (3.44–8.38)***	4.88 (3.09–7.71)***	5.72 (3.65–8.95)***	5.22 (3.29–8.27)***
aMMP-8/NTP	3.90 (2.19–6.91)***	3.38 (1.94–5.92)***	4.33 (2.14–8.76)***	3.61 (1.83–7.13)***	1.61 (1.34–1.94)***		1.64 (1.37–1.98)***	
Shanghai study	*N* = 386 vs. 4		*N* = 385 vs. 5		*N* = 208 vs. 182		N = 208 vs. 182	
Male	Insufficient observations for valid comparisons				2.67 (1.72–4.16)***	2.49 (1.56–3.98)***	2.67 (1.72–4.16)***	2.49 (1.56–3.98)***
Age					1.04 (1.02–1.05)***		1.04 (1.02–1.05)***	
Current smokers					1.49 (0.64–3.50)		1.49 (0.64–3.50)	
Systemic disease					1.37 (0.89–2.11)		1.37 (0.89–2.11)	
aMMP-8 positive test					3.45 (2.24–5.34)***		3.45 (2.24–5.34)**	
aMMP-8/NTP					3.19 (2.16–4.70)***	3.16 (2.12–4.72)***	3.19 (2.16–4.70)***	3.16 (2.12–4.72)***

(d) Periodontitis grade

	Grade B versus grade A		Grade C versus grade A		Grade C versus grade B		Grade C versus grade A + grade B	
	Crude	Adjusted	Crude	Crude	Crude	Adjusted	Crude	Adjusted
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Hong Kong study								
Male	0.90 (0.34–2.37)		2.18 (0.83–5.72)		2.42 (1.46–4.02)*		2.39 (1.46–3.89)***	1.73 (1.01–2.98)*
Age	1.04 (1.01–1.07)*	1.04 (1.01–1.08)*	1.06 (1.02–1.10)**	1.06 (1.03–1.10)**	1.02 (0.99–1.04)		1.03 (1.01–1.05)*	1.03 (1.00–1.05)*

(Continues)

TABLE 4 | (Continued)

(d) Periodontitis grade

	Grade B versus grade A		Grade C versus grade A		Grade C versus grade B		Grade C versus grade A + grade B	
	Crude	Adjusted	Crude	Crude	Crude	Adjusted	Crude	Adjusted
Variables	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Current smokers	/		/		7.76 (1.70–35.41)**	11.51 (2.48–55.44)**	8.97 (1.97–40.88)**	12.21 (2.54–58.80)**
Systemic disease	0.91 (0.28–2.92)		1.36 (0.42–4.33)		1.49 (0.81–2.75)		1.47 (0.82–2.65)	
aMMP-8 positive test	0.91 (0.36–2.32)		3.29 (1.28–8.48)*	4.10 (1.47–11.39)**	3.61 (2.17–6.01)***		3.56 (2.18–5.84)***	
aMMP-8/ NTP	0.82 (0.60–1.14)		1.37 (0.97–1.94)		1.78 (1.43–2.22)***	1.84 (1.47–2.31)***	1.65 (1.36–2.00)***	1.65 (1.35–2.02)***
Shanghai study								
Male	4.97 (1.45–17.07)*	5.46 (1.55–19.22)**	21.38 (5.61–81.41)***	44.94 (2.16–936.75)*	4.30 (2.20–8.42)***		6.31 (3.31–12.03)***	2.76 (1.25–6.11)*
Age	1.06 (1.00–1.12)*		1.30 (1.16–1.45)***	1.32 (1.12–1.56)***	1.10 (1.07–1.13)***	1.10 (1.07–1.13)***	1.12 (1.09–1.15)***	1.11 (1.07–1.14)***
Current smokers	0.38 (0.07–2.08)		4.97 (1.04–23.71)*		12.97 (4.41–38.17)***	11.78 (3.41–40.63)***	12.08 (5.02–29.08)***	6.8 (2.20–21.08)***
Systemic disease	1.19 (0.51–2.76)		2.22 (0.85–5.78)		1.87 (1.00–3.50)*		2.56 (1.41–4.63)**	
aMMP-8 positive test	9.79 (2.86–33.49)***	10.37 (3–35.91)***	15.05 (4.01–56.44)***	55.05 (2.34- > 999.999)*	1.54 (0.82–2.90)		3.16 (1.72–5.79)***	2.62 (1.21–5.68)*
aMMP-8/ NTP	7.94 (2.13–29.55)**		11.57 (2.72–49.31)***		1.51 (1.15–1.99)**		2 (1.52–2.62)***	

Note: After adjustment for gender, age, smoking and systemic condition, *p*-value and OR are provided when the variables remain in the final model; OR, odds ratio; 95% CI, confidence interval of 95%; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$; aMMP-8/NTP, total aMMP-8 concentration divided by the number of teeth present. PPD, probing pocket depth; bleeding pocket, PPD ≥ 4 mm or PPD ≥ 6 mm concurrent with bleeding on probing.

TABLE 5 | Diagnostic utility of the aMMP-8 test results and demographic factors to discriminate periodontitis (the threshold value for the aMMP-8 test is set at 20 ng/mL).

	Periodontitis (from the whole population)			Stages I/II periodontitis (from the non-periodontitis)			Stages III/IV periodontitis (from the whole population)			Stage IV periodontitis (from the whole population)		
Variables	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Hong Kong study												
Male									X			
Age			X			X			X			X
Current smokers									X			X
Systemic disease												
aMMP-8 positive test	X		X	X		X	X		X	X		
aMMP-8/NTP		X			X			X			X	X
Performance												
AUROC (95% CI)	0.661 (0.603–0.720)	0.768 (0.716–0.820)	0.904 (0.867–0.942)	0.586 (0.498–0.673)	0.636 (0.551–0.721)	0.828 (0.767–0.889)	0.651 (0.596–0.706)	0.762 (0.714–0.810)	0.855 (0.818–0.892)	/	0.733 (0.661–0.804)	0.809 (0.750–0.868)
Cut-off value	20 ng/mL	0.4317 ng/mL	0.758	20 ng/mL	0.4416 ng/mL	0.385	20 ng/mL	0.4317 ng/mL	0.472	20 ng/mL	0.4152 ng/mL	0.068
Sensitivity	47.6%	90.1%	84.6%	32.4%	47.3%	79.5%	52.8%	76.9%	86.8%	/	95.4%	95.4%
Specificity	84.7%	78.6%	82.7%	84.7%	76.5%	74.5%	73.3%	66.3%	67.3%	/	42.2%	53.5%
PPV	90.1%	74.4%	93.4%	61.5%	60.3%	69.9%	74.2%	73.8%	76.7%	/	17.2%	20.6%
NPV	35.6%	73.3%	64.8%	62.4%	65.8%	83.0%	57.1%	69.4%	80.4%	/	98.6%	98.9%
Shanghai study												
Male			X									
Age			X			X			X			X
Current smokers												

(Continues)

TABLE 5 | (Continued)

	Periodontitis (from the whole population)			Stages I/II periodontitis (from the non-periodontitis)			Stages III/IV periodontitis (from the whole population)			Stage IV periodontitis (from the whole population)		
Variables	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Systemic disease			X			X						
aMMP-8 positive test	X			X			X		X	X		
aMMP-8/NTP		X	X		X	X		X			X	X
Performance												
AUROC (95% CI)	0.669 (0.626–0.714)	0.769 (0.721–0.818)	0.805 (0.762–0.849)	0.631 (0.582–0.679)	0.719 (0.663–0.777)	0.73 (0.673–0.786)	0.681 (0.624–0.738)	0.784 (0.732–0.836)	0.937 (0.903–0.972)	0.725 (0.629–0.822)	0.853 (0.771–0.935)	0.951 (0.921–0.98)
Cut-off value	20 ng/mL	0.4 ng/mL	0.590	20 ng/mL	0.4 ng/mL	0.549	20 ng/mL	0.826 ng/mL	0.329	20 ng/mL	1 ng/mL	0.022
Sensitivity	50.4%	77.1%	75.2%	42.5%	70.2%	66.9%	67.9%	67.9%	85.2%	82.4%	88.2%	100%
Specificity	83.6%	64.8%	77.3%	83.6%	64.8%	75.8%	68.3%	72.5%	93.5%	62.7%	71.6%	81.2%
PPV	86.3%	81.8%	87.2%	78.6%	73.8%	79.6%	35.9%	39.3%	77.5%	9.2%	12.4%	19.5%
NPV	45.1%	58%	60.4%	50.7%	60.6%	61.8%	89%	89.6%	96%	98.7%	99.3%	100%

Note: Model 1 was a crude analysis of a positive aMMP-8 test; Model 2 was a crude analysis of aMMP-8/NTP; Model 3 was the selection of the best significant subset of variables using the demographic factors and Models 1–2. aMMP-8/NTP, the total aMMP-8 concentration divided by the number of teeth present; non-periodontitis = periodontal health + gingivitis, /: fail to detect the condition.
Abbreviations: AUROC, area under receiver operator characteristic curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

TABLE 6 | Diagnostic utility of the aMMP-8 test results and demographic factors to discriminate different grades and extents of periodontitis (the threshold value for the aMMP-8 test is set at 20 ng/mL).

	Periodontitis grade C (from periodontitis grade A and grade B)			Generalised periodontitis (from localised periodontitis)		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Hong Kong study						
Male			X			
Age			X			X
Current smokers			X			
Systemic disease						
aMMP-8 positive test	X			X		
aMMP-8/NTP		X	X		X	X
Performance						
AUROC (95% CI)	0.654 (0.589–0.718)	0.736 (0.678–0.793)	0.764 (0.707–0.821)	0.570 (0.503–0.638)	0.662 (0.598–0.725)	0.708 (0.647–0.768)
Cut-off value	20 ng/mL	1.4599 ng/mL	0.382 0.382	20 ng/mL	0.3964 ng/mL	0.610 0.610
Sensitivity	65.3%	55.4%	72.7%	53.2%	47.3%	58.5%
Specificity	65.5%	80.6%	75.0%	60.9%	76.5%	74.6%
PPV	58.1%	67.7%	68.2%	66.9%	60.3%	77.5%
NPV	72.0%	71.1%	78.9%	46.7%	65.8%	54.5%
Shanghai study						
Male			X			
Age			X			X
Current smokers			X			
Systemic disease						
aMMP-8 positive test	X			X		
aMMP-8/NTP		X			X	
Performance						
AUROC (95% CI)	0.582 (0.507–0.656)	0.648 (0.565–0.73)	0.893 (0.851–0.935)	0.546 (0.484–0.609)	0.595 (0.523–0.666)	0.697 (0.632–0.762)
Cut-off value	20 ng/mL	1 ng/mL	0.143	20 ng/mL	1.222 ng/mL	0.660
Sensitivity	63.5%	61.5%	92.3%	53%	72.9%	72.3%

(Continues)

TABLE 6 | (Continued)

	Periodontitis grade C (from periodontitis grade A and grade B)			Generalised periodontitis (from localised periodontitis)		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Specificity	52.9%	63.8%	75.2%	56.3%	42.7%	60.4%
PPV	25%	29.6%	48%	67.7%	68.8%	75.9%
NPV	85.4%	87%	97.5%	40.9%	47.7%	55.8%

Note: Model 1 was a crude analysis of a positive aMMP-8 test; Model 2 was a crude analysis of aMMP-8/NTP; Model 3 was the selection of the best significant subset of variables using the demographic factors and Models 1–2. aMMP-8/NTP, the total aMMP-8 concentration divided by the number of teeth present; non-periodontitis = periodontal health + gingivitis.
Abbreviations: AUROC, area under receiver operator characteristic curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

4 | Discussion

4.1 | Main Findings and Interpretation

The present studies and their incorporation into a recent meta-analysis (Wei et al. 2024) indicate that there is moderate certainty that a positive aMMP8 test result in an oral rinse sample predicts periodontitis with low to moderate sensitivity, moderate to high specificity, and moderate accuracy. Sensitivity refers to the ability of a test to designate an individual with the disease as positive. The specificity of a test is its ability to assign an individual who does not have the disease as negative. The observed low to moderate sensitivity implies that the test cannot 'rule out' periodontitis because it is expected to be positive in only 59% of subjects with the disease (95% CI: 42%–75%). Periodontitis will remain undetected in the diseased subjects with a false negative test. Moderate to high specificity means that the test can 'rule in' periodontitis because it is expected to correctly classify 82% of people with periodontitis as positive (95% CI: 68%–93%). False positives require additional testing to exclude the disease. The hierarchical summary ROC-AUC is the credible accuracy estimate (HSROC-AUC of 0.77, 95% CrI: 0.74–0.81) and indicates that the test has moderate inherent validity. It is well recognized, however, that clinicians have difficulties applying test accuracy data to clinical decision-making (Whiting et al. 2015). Pre– post-test probabilities have been suggested to illustrate the consequences of performing a test. Among subjects with a positive test, 81% had periodontitis (PPV). A positive test can thus be interpreted as a reasonable indication that the subject has periodontitis, but further assessment is required to confirm this. The 19% misclassification may be acceptable given the nature of the disease and the relative accessibility for a dental examination to confirm or exclude the disease. Conversely, 64% of subjects with a negative test did not have periodontitis. The resulting 36% false negative rate, however, is significant and indicates that a negative test does not rule out the presence of periodontitis. The wide intervals for the PPV and NPV (Table 7) further highlight the limitations in correctly interpreting the test result.

It is essential to highlight that these results apply to using the aMMP8 test to screen or diagnose the periodontitis status of untreated subjects. They do not contribute to diagnostic questions about the accuracy of biomarkers in predicting disease progression or treatment response (Teles et al. 2024).

The addition of the present studies to the SROC curve of the Bayesian meta-analysis significantly narrowed the credible region describing the accuracy of the aMMP8 test (Figure 1). The moderate certainty of the accuracy estimates enables, for the first time, a meaningful discussion about the applicability of this aMMP8 POC-ORT. The high false negative rate (low NPV) advises against using the test when alternative more sensitive options are available, like in dental clinical settings. It can be used as a screening test in a medical or periodontitis self-detection context, and it may benefit the subjects with a positive test result. However, evidence of benefits in these settings is still at the proof-of-principle stage, with emerging evidence for the integrated care of the periodontitis–diabetes co-morbidity (Heikkinen et al. 2023; Nijland et al. 2021).

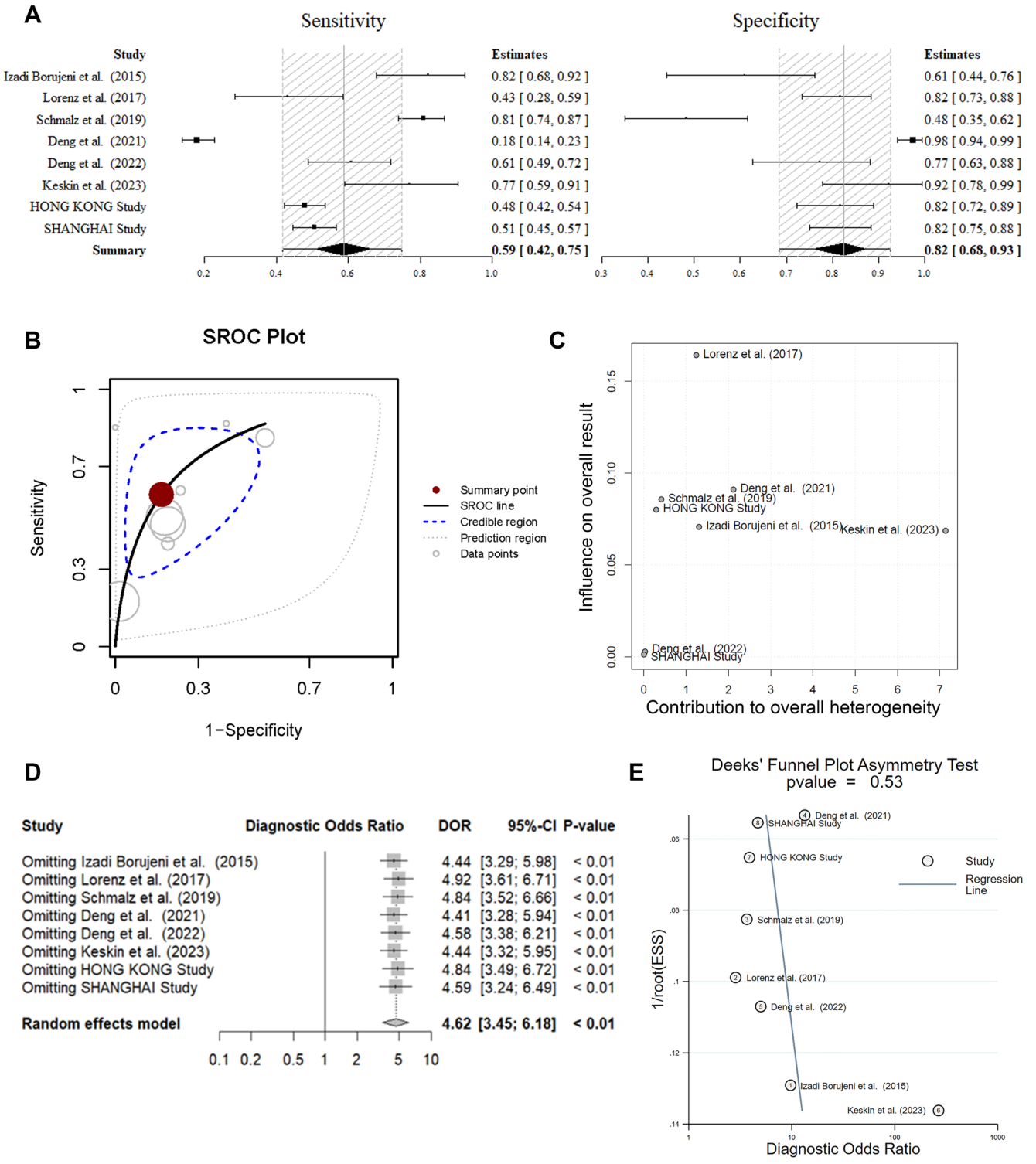

FIGURE 1 | Updated meta-analysis of the accuracy of aMMP-8 POC-ORT to detect periodontitis. The update of the meta-analysis by Wei et al. (2024), including the present two studies labelled as Hong Kong and Shanghai. Panel (A) shows the Forrest plot of sensitivity and specificity of aMMPT-8 POCT to predict periodontitis. The diamond shows the summary estimate and the 95% confidence interval. Some inconsistency between the included studies is evident. Panel (B) illustrates the hierarchical summary receiver operating characteristic (SROC) plot. It is a measure of the inherent validity of the test. The size of the circles showing the data points represents the sample size of the individual studies. The red circle shows the summary point, and the black line shows the calculated SROC curve. The blue dashed line defines the credible region for the accuracy estimate: The Bayesian estimate of the 95% credible interval. The Baujat plot (Panel C) shows the contribution of each study to the overall heterogeneity and the overall result. Panel (D) summarizes the diagnostic odds ratios and the stability of the values obtained by omitting one study. Lastly, Panel E is the Deeks funnel plot showing the lack of evidence of a publication bias.

TABLE 7 | Certainty of evidence.

Summary of findings table								
Population/setting: Adults with periodontitis								
New test: aMMP-8 POC-ORT								
Reference test: Full periodontal examination								
Purpose of the new test: Parallel or combined								
No. of studies/ sample size	**Study design**	**Risk of bias**	**Indirect evidence**	**Inconsistency**	**Imprecision**	**Publication bias**	**Effect measure**	**Certainty of the evidence**
8/2048	5 cross-sectional 3 case control	↓	/	Early studies	Some	/	Sensitivity: 0.59 (95% CI: 0.42–0.75) Specificity: 0.82 (95% CI: 0.68–0.93) HSROC-AUC: 0.77 (95% CrI: 0.74–0.81) NPV: 0.64 (95% CI: 0.45–0.80) PPV: 0.81 (95% CI: 0.61–0.92)	⨁⨁⨁◯ Moderate

Note: GRADE Working Group grades of evidence: High certainty: we are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.
Abbreviations: HSROC-AUC, hierarchical summary receiver operating characteristic area under the curve; NPV, negative predictive value; PPV, positive predictive value.

4.2 | Strengths and Limitations

The presented original studies are notable for the remarkable consistency of the diagnostic accuracy results across two different Chinese populations: one drawn from a random sample, and the other from consecutive subjects reporting for dental care. The populations differed greatly in age, co-morbidities, extent of periodontal inflammation, and periodontitis stage and grade distribution. Both samples, however, had a high prevalence of periodontitis. A dose effect was also observed: the test better predicted more severe disease.

The gingival crevicular fluid contains biomarkers originating in the marginal periodontium because of inflammation or tissue breakdown and accumulates them in the oral cavity. Saliva samples or purposely collected timed oral rinses (oral fluids) have been used to obtain an overall fingerprint of the biomarkers of interest across the whole dentition. The original concentration within the tissue or in the gingival sulcus/pocket, the gingival crevicular fluid's production rate, and the number of teeth presenting inflammatory changes/periodontal breakdown affect the concentrations of biomarkers in oral fluids. The present studies confirmed that correcting for the number of teeth present improves accuracy estimates and points to the inherent limitations in the diagnostic performance of oral fluid samples. This limitation also becomes apparent in the analyses of a recent longitudinal study: better accuracy was observed in subjects with more progressing sites in the dentition (Teles et al. 2024). In addition, the tested inflammatory biomarkers are present, albeit at different concentrations, in the full periodontal health–disease spectrum. Hence, it is impossible to discriminate the contribution to the biomarker concentration in the oral fluid sample of multiple teeth with gingivitis from that of a localized periodontitis lesion. Subjects in the population have a unique profile of lesions in their dentitions. However, focusing on site-specific crevicular fluid sampling is impractical, particularly for initial screening or diagnosis.

Moreover, the clinical attachment level reference standard has limitations. The critical one is its imprecision in discriminating gingivitis from stage I (incipient) periodontitis. This issue was well recognized by the proponents of the 2017 Classification system and by the workshop consensus. The original concept was that a biomarker would enable the discrimination between periodontitis and gingivitis. Another limitation comes from the generalized/localized periodontitis designation set at the stage-defining level (Sanz et al. 2020). Periodontitis extent may be a better designation than the current case definition, which may bias the test performance. More work is needed to address this potential confounder.

These challenges need to be recognized when interpreting the performance of an oral fluid diagnostic test. They point to an inherent limitation that may prevent an analytically accurate oral fluid test from showing high sensitivity, specificity, and diagnostic accuracy. In such a scenario, the test's clinical applicability may be best discussed based on the consequences of misclassification.

The data also confirm previous observations that incorporating demographic factors in multivariate models improved accuracy, particularly sensitivity. Age was the most consistent attribute that improved the accuracy of the aMMP8 test for periodontitis prediction. However, previous research on an independent population showed that confusion matrices of diagnostic models incorporating age risked missing advanced-stage periodontitis in younger individuals (Deng et al. 2023). Low self-reported prevalence of recognized risk factors (smoking and diabetes) does not allow proper evaluation of their significance in these populations. The applicability of the test to populations with a high prevalence of smoking or diabetes remains unclear (Wei et al. 2024).

4.3 | Conclusion

The moderate certainty of the diagnostic accuracy estimates of aMMP-8 POC-ORT in treatment-naïve subjects enables an evidence-based assessment of the test's characteristics and, thus, its potential applicability. The low to moderate sensitivity speaks against using the test in a clinical dental setting instead of a clinical or radiographic periodontal examination. The moderate to high PPV reveals its potential utility for self-detection or co-management of periodontitis in a medical setting and its incorporation in multi-test diagnostics. Further investigations are highly warranted.

Author Contributions

M.S.T. conceived this work, wrote the study protocols, and drafted the report. L.J. and M.S.T. designed the study, secured the funding, supervised the work, the and interpreted the data. Y.L., J.C.K.K., J.S., X.W. and S.L.T.L. collected the data. H.L. and G.P. ensured quality control of data collection and supervised the work. Y.L. and X.Z. analysed the data. K.D. resolved case diagnosis queries and performed the updated meta-analysis. All authors contributed to the revision of the draft and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.