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Clinical Features Associated With Periodontal Case Misclassification by an Active Matrix Metalloproteinase-8 Point-of-Care Oral Rinse Test

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ABSTRACT

Background: The diagnostic performance of active matrix metalloproteinase-8 (aMMP-8) point-of-care oral rinse test (POC-ORT) for periodontitis needs to be improved because of its relatively low sensitivity. This study attempted to identify the clinical features associated with the misclassification of periodontal cases by the test.

Method: This work consisted of two cross-sectional diagnostic accuracy studies involving a representative cohort in Hong Kong, China, and a convenience sample of subjects in Shanghai, China. The outcomes of the aMMP-8 POC-ORT (index test) were compared to the case definitions of the 2017 World Workshop on the classification of periodontal health status (reference test). The analysis reports the diagnostic accuracy parameters, the Youden index and correlations between the aMMP-8 test outcomes and clinical features.

Results: In this study, 384 and 390 subjects were enrolled in Hong Kong and Shanghai, respectively. The conventional 20 ng/mL threshold failed to detect more than 50% of early-stage (I/II) cases. The positive POC-ORT results were significantly correlated with the number of sites with bleeding on probing (BOP), probing pocket depth (PPD) ≥ 4 mm, PPD ≥ 5 mm, PPD ≥ 4 mm and BOP, PPD ≥ 5 mm and BOP and clinical attachment loss. The adjusted odds ratio (OR) for a positive test increased with the number of sites with PPD ≥ 5 mm: 1.092 (95% CI: 1.063–1.127, $p < 0.001$) for the Hong Kong sample and 1.074 (95% CI: 1.050–1.100, $p < 0.001$) for the Shanghai sample, suggesting a higher likelihood of a positive result with each additional pocket ≥ 5 mm. The same was observed for PPD ≥ 5 mm with BOP: 1.093 (95% CI: 1.064–1.129, $p < 0.001$) and 1.077 (95% CI: 1.052–1.105, $p < 0.001$). Notably, the false-negative cases were characterised by a significantly smaller number of periodontal pockets, BOP percentages and bleeding pockets than the true-positive ones.

Mengning Bi and Yu Xie contributed equally to this study.

Conclusions: The false-negative cases, which are responsible for the observed low sensitivity of the POC-ORT, showed more localised periodontal inflammation and pocketing. As the oral rinse test measures the overall level of aMMP-8 cleared by the gingival crevicular fluid from various lesions throughout the whole dentition, localised periodontitis appears more difficult to detect. Additional studies are needed to optimise the sampling strategy and set the cut-off value of a positive test.
Trial Registration: The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB Approval: UW22-132; trial registration: NCT03928080) and the Shanghai Ninth People's Hospital Ethics Committee (IRB Approval: SH9H-2021-T408-3; trial registration: NCT05513599)

1 | Introduction

Periodontitis is characterised by inflammation linked to a dysbiotic biofilm and an imbalance in the host immune response, leading to periodontal attachment loss and tooth loss. This condition ranks among the top global public health challenges and is associated with a higher susceptibility to systemic diseases. It has significant socio-economic implications and profoundly affects an individual's oral and overall well-being (Tonetti et al. 2017).

The diagnosis of periodontitis is based on patient history as well as clinical and radiographic examinations. These include assessing the clinical attachment level, probing depths, degree of gingival inflammation and alveolar bone loss. These diagnostic procedures require multiple manual recordings and highly skilled professional examiners.

Because of the limitations of traditional methods, researchers are exploring point-of-care technologies that make use of oral fluids. These technologies can be used by non-dental professionals or patients themselves to enhance the detection and monitoring of periodontitis (Räisänen et al. 2023). In this context, saliva and oral rinses are preferred for screening because of their non-invasiveness as well as the rapid collection and rich content of periodontal biomarkers (Lähteenmäki et al. 2023; Melguizo-Rodríguez et al. 2020).

Inflammation-induced matrix metalloproteinases (MMPs) are key mediators of periodontal tissue destruction, degrading major components of the extracellular matrix and basement membrane (Nunn 2003; Sorsa et al. 2016). Matrix metalloproteinase-8 (MMP-8) is the main collagenase found in periodontal tissues and plays a crucial role in the pathological process of periodontitis. It is closely associated with the severity of periodontal disease (Ingman et al. 1996; Javed et al. 2014; Marcaccini et al. 2010).

Increased levels of MMP-8, especially its activated form (aMMP-8), have been found in oral fluids from subjects with periodontitis (Gul et al. 2020; Sorsa et al. 2006; Wahlgren et al. 2002). As a result, aMMP-8 is considered one of the most promising diagnostic biomarkers for periodontitis and is commonly measured in the gingival crevicular fluid (GCF) or saliva (Arias-Bujanda et al. 2019, 2020). The primary source of aMMP-8 in the oral cavity is GCF, and the concentration of aMMP-8 in GCF is much higher than in other oral fluids (Ramenzoni et al. 2021). Additionally, in standardised oral rinse samples that include a pre-rinse step, samples collected from oral rinses have been shown to accurately reflect the total aMMP-8 concentrations in the GCF, providing a comprehensive assessment of the overall oral inflammatory status (Gangbar et al. 1990; Leppilahti et al. 2011).

Recently, we quantitatively summarised the diagnostic accuracy of trials assessing aMMP8, commercially available as a point-of-care oral rinse test (POC-ORT), for screening and diagnosing treatment-naïve subjects (Li et al. 2025). Using a threshold of 20 ng/mL, our meta-analysis revealed that the test has a moderate certainty of 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 area under the receiver operating characteristic (AUROC) of 0.77 (95% CrI: 0.74–0.81). The notably low to moderate sensitivity remains a significant barrier to its application. This is reflected in the consensus report of the recent European Federation of Periodontology workshop on periodontal diagnosis (Herrera et al. 2025). Additionally, limited research has explored the factors that may contribute to this observed low sensitivity. In this paper, we focus on analysing the clinical features associated with misclassification, which is a crucial step in understanding the limitations and designing and refining future biomarker tests for assessing periodontal health.

This study aimed to (i) investigate the correlation between the aMMP-8 POC-ORT test results and clinical periodontal parameters, including the number of sites with bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL); (ii) examine the relationship between the dichotomised aMMP-8 POC-ORT test results, the aMMP-8 value, the ratio of aMMP-8 to the number of teeth present (aMMP-8/NTP) and the number or proportion of diseased sites in the dentition; and (iii) further test the diagnostic capability of the aMMP-8 POC-ORT, investigate the reasons for the test's low sensitivity, identify the characteristics of false-negative (FN) patients and optimise the context and scenarios for using this test to enhance evidence-based, individualised screening strategies.

2 | Methods

2.1 | Study Design and Populations

Two cross-sectional prospective diagnostic accuracy studies were conducted to evaluate the performance of aMMP-8 POCT (Index Test) against full-mouth clinical periodontal examination and history by specialists (Reference Test) at the Prince Philip Dental Hospital (Hong Kong) and the Ninth People's Hospital (Shanghai) from April 2022 to April 2024. The study design, population and sample size estimation have been previously reported (Li et al. 2025). Raters for the index and reference tests were different and unaware of the results of the other test.

The studies were undertaken following the current Declaration of Helsinki, with written informed consent from all participants. This report adhered to the STARD guidelines (Cohen et al. 2016).

2.2 | Oral Rinse aMMP-8 Point-of-Care Index Test

A commercial aMMP-8 POCT system (PerioSafe PRO, Dentognostics GmbH) and its digital analysis device (ORALyzer, Dentognostics) were used (Li et al. 2025). The test is a lateral flow immunoassay designed to quantify aMMP-8 concentrations. The test used filtered 30-s oral rinse. According to the manufacturer's recommendation, diagnostic performance was evaluated using a cut-off value of 20 ng/mL, with any aMMP-8 levels below the detection threshold of 10 ng/mL being treated as 10 ng/mL for statistical analysis. Quantitative results of the aMMP-8 test were also related to the number of teeth present (NTP), as obtained by dividing the total concentration by NTP (aMMP-8/NTP).

2.3 | Periodontal Examination and Case Definition—Reference Standard

The outcomes of full-mouth periodontal examinations were taken as the reference standard for diagnosing periodontal health, gingivitis and different stages of periodontitis. Clinical assessment involved measuring PPD, BOP and CAL at six sites per tooth using a periodontal probe (UNC-15, Hu Friedy, Chicago). Furcation involvement (FI), tooth mobility and tooth loss due to periodontitis were also evaluated. Examiners were trained and calibrated, achieving a kappa (κ) value > 0.85 for PPD and CAL measurements. Duplicate measurements were taken for every 30 subjects, maintaining the κ values above 0.85. Demographic, smoking and medical histories were also collected.

The 2017 classification of periodontal diseases was used to diagnose various periodontal conditions (Chapple et al. 2018; Papapanou et al. 2018; Tonetti et al. 2018; Tonetti and Sanz 2019; Trombelli et al. 2018). Unaware of the aMMP-8 test results, the examiners employed the algorithm that Tonetti and Sanz (2019) suggested to diagnose each case.

2.4 | Statistical Analysis

Statistical analyses were performed using SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). The level of significance was set at 0.05 for all tests. Comparisons of differences in categorical variables were made using the chi-squared test and Fisher's exact test. As previously described, sensitivity and specificity were defined to be low (< 60%), moderate (60%–79%) or high (> 80%) (Nelson et al. 2001). The Youden index (sensitivity + specificity − 1) assesses the diagnostic accuracy by combining sensitivity and specificity. Differences in continuous variables among patient groups were tested using the nonparametric Kruskal–Wallis test. For multiple-group comparisons, pairwise comparisons between the groups were conducted using a Bonferroni method to adjust the significance level if group differences were indicated ($p < 0.05$). Spearman's rank correlation coefficient (ρ) was used for the analysis of correlation of aMMP8 levels and aMMP8/NTP with periodontal clinical parameters. Simple and multiple linear regression analysed the association of aMMP-8 levels and aMMP-8/NTP with clinical parameters, while binary logistic regression evaluated the association of dichotomised aMMP-8 POC-ORT results with clinical parameters. The Mann–Whitney U test was used to compare two groups. Since a large number of test results were below the detection level, two data processing methods were employed to assess whether recording samples with undetectable concentrations as 10 ng/mL influenced the robustness of the results in subsequent analyses: (1) randomly assigning values between 0 and 10 ng/mL to samples currently recorded as 10 ng/mL, with this process repeated three times, and (2) treating aMMP-8 concentrations as an ordinal variable, where samples in the 0–10 ng/mL range were assigned a value of 1 and each subsequent 10 ng/mL range was assigned an increasing level.

3 | Results

A total of 384 participants were enrolled in Hong Kong and 390 in Shanghai. Table S1 presents the demographic characteristics, risk factor profiles and periodontal parameters for each group. There were no missing data for the index or reference tests. In addition, significant differences in age, sex and clinical parameters were observed across case diagnoses in both studies. No adverse events related to the index test or reference standard were observed.

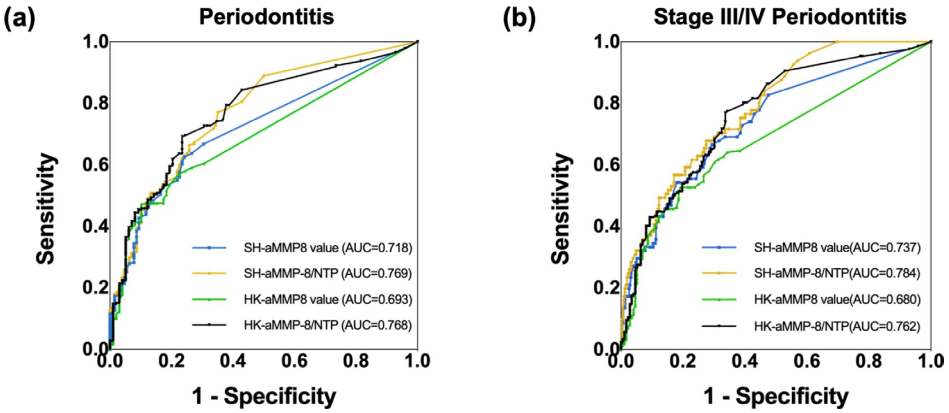

FIGURE 1 | Receiver operating characteristic (ROC) curves of aMMP-8 in oral rinses for the detection of periodontitis. (a) ROC curves for detecting periodontitis from the whole population. (b) ROC curves for predicting stage III/IV periodontitis from the whole population. AUC, area under the curve; HK, Hong Kong study; SH, Shanghai study.

TABLE 1 | Accuracy of the dichotomised aMMP-8 POC-ORT to discriminate various periodontal case definitions.

Diagnostic accuracy measures	Periodontal case definitions							
				Stages of periodontitis				*p*
	Periodontal disease	Gingivitis	Periodontitis	I	II	III	IV	P (I–IV)/NP
Hong Kong study	*N* = 360	*N* = 74	*N* = 286	*N* = 12	*N* = 62	*N* = 169	*N* = 43	
Threshold level = 20 ng/mL								
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
Test negative (*n*/%)	210 (58.3%)	60 (81.1%)	150 (52.4%)	8 (66.7%)	42 (67.7%)	79 (46.7%)	21 (48.8%)	
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%	
Threshold level = 24 ng/mL								
Test positive (*n*/%)	144 (40.0%)	9 (12.2%)	135 (47.2%)	4 (33.3%)	19 (30.6%)	90 (53.3%)	22 (51.2%)	< 0.001
Test negative (*n*/%)	216 (60.0%)	65 (87.8%)	151 (52.8%)	8 (66.7%)	43 (69.4%)	79 (46.7%)	21 (48.8%)	
Sensitivity	40.0%	12.2%	47.2%	33.3%	30.6%	53.3%	51.2%	
Specificity	95.8%	56.1%	89.8%	62.1%	60.9%	74.4%	63.9%	
Shanghai study	*N* = 380	*N* = 118	*N* = 262	*N* = 153	*N* = 28	*N* = 64	*N* = 17	
Threshold level = 20 ng/mL								
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
Test negative (*n*/%)	227 (59.7%)	97 (82.2%)	130 (49.6%)	89 (58.2%)	15 (53.6%)	23 (35.9%)	3 (17.6%)	
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%	
Threshold level = 13 ng/mL								
Test positive (*n*/%)	195 (51.3%)	31 (26.3%)	164 (62.6%)	87 (56.9%)	15 (53.6%)	47 (73.4%)	15 (88.2%)	< 0.001
Test negative (*n*/%)	185 (48.7%)	87 (73.70%)	98 (37.4%)	66 (43.1%)	13 (46.4%)	17 (26.6%)	2 (11.8%)	
Sensitivity	51.3%	26.3%	62.6%	56.9%	53.6%	73.4%	88.2%	
Specificity	100%	39.7%	75.8%	54.4%	50.3%	54.6%	51.7%	

Note: Periodontal disease = gingivitis + periodontitis. Chi-squared tests were used to assess differences between groups. Performance refers to discriminating periodontal disease or gingivitis from periodontal health and discriminating periodontitis (Stages I to IV) from non-periodontitis. For the two studies, two thresholds were tested: the one recommended by the test manufacturer, and the other identified by the Youden index to maximise the sensitivity and specificity of the test.

Abbreviations: G, gingivitis; H, periodontal health; I, stage I periodontitis; II, stage II periodontitis; III, stage III periodontitis; IV, stage IV periodontitis; NP, non-periodontitis; P, periodontitis.

3.1 | Effect of Test Threshold on the Diagnostic Performance of the aMMP-8 POC-ORT

As shown in Figure 1, aMMP-8 POC-ORT showed comparable screening capabilities for both total periodontitis and stage III/IV periodontitis. In both the Hong Kong and Shanghai studies, the combination of aMMP-8 and NTP outperformed aMMP-8 alone in identifying total and severe periodontitis. With a threshold of 20 ng/mL, the aMMP-8 test showed a sensitivity and specificity of 0.504 and 0.836 in the Shanghai study, and 0.476 and 0.827 in the Hong Kong study, respectively. Notably, ROC curve analysis revealed population-specific optimal thresholds: the Shanghai study achieved the highest Youden index at 13 ng/mL with a sensitivity of 0.626 and a specificity of 0.758, whereas the Hong Kong study showed peak diagnostic efficiency at 24 ng/mL with a sensitivity of 0.472 and a specificity of 0.898. Table 1 presents the discriminative performance of the dichotomised aMMP-8 POC-ORT results at various threshold values for identifying different periodontal health statuses. Owing to the limitations in detection accuracy of quantitative aMMP-8 measurements, we were unable to determine the exact concentrations for cases with aMMP-8 levels below 10 ng/mL. Therefore, we have recorded all samples with concentrations below 10 ng/mL as 10 ng/mL for the purpose of analysis, which is a commonly used approach. To assess the robustness of our findings, we employed two additional data processing methods to address the potential impact of this limitation. We found that the results obtained from all three methods were consistent (see Table S2).

3.2 | Associations of the Quantitative aMMP-8 Results With Periodontal Health Status

The quantitative aMMP-8 levels and aMMP-8/NTP levels, according to case definitions, are illustrated in Figure 2. Significant differences were observed in the median levels of aMMP-8 between periodontal health or gingivitis and stages III or IV periodontitis. Compared to aMMP-8 alone, aMMP-8/NTP showed differences in a broader range of periodontal case definitions. These findings suggest that aMMP-8/NTP shows a more pronounced difference across various periodontal health statuses. Furthermore, it was noted that, except for stages III and IV periodontitis, the median aMMP-8 levels in periodontal health, gingivitis and stages I and II periodontitis were all below the manufacturer's recommended threshold of 20 ng/mL. This suggests that more than half of the patients with stage I and II periodontitis may be misdiagnosed, which is an issue of concern that should be addressed.

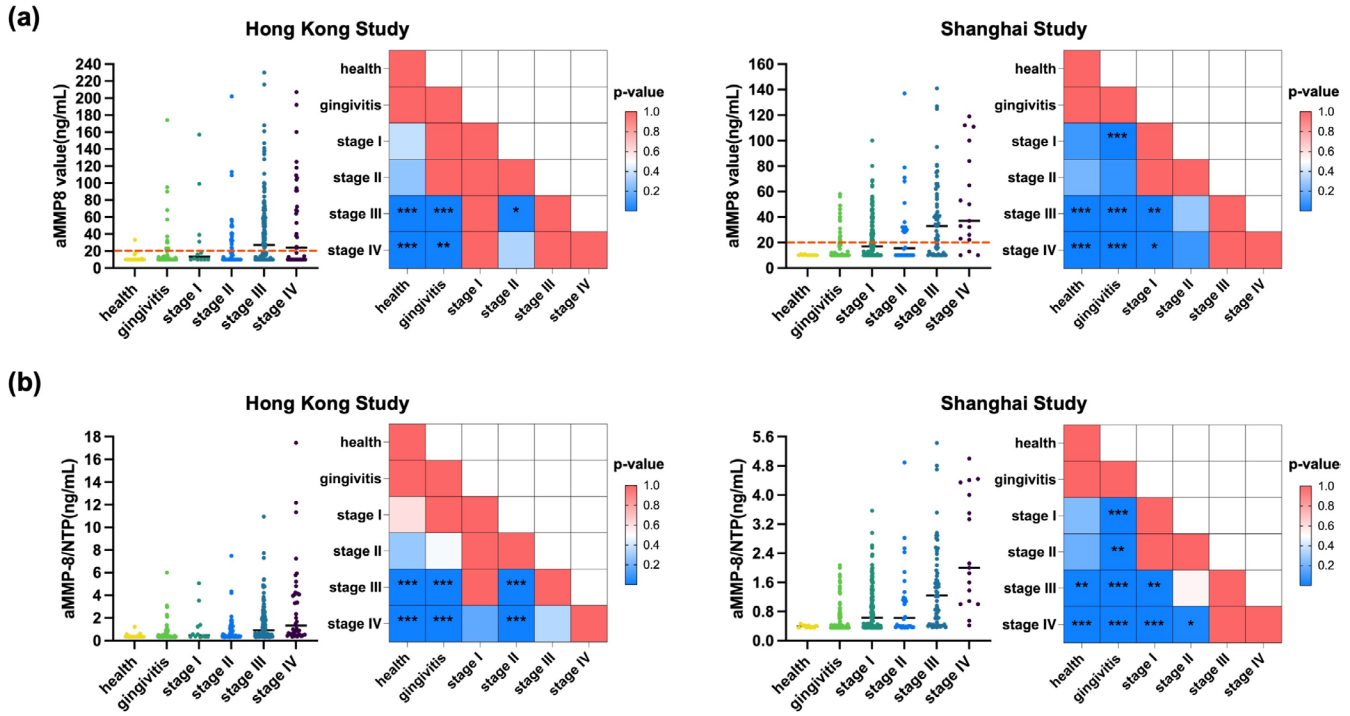

FIGURE 2 | aMMP-8 concentrations in oral rinse across the spectrum of periodontal case definitions. The figure shows quantitative aMMP8 and aMMP-8/NTP levels in oral rinses by periodontal case definitions in the Hong Kong (left column) and Shanghai (right column) studies. Each image pair illustrates the observed median concentrations across the different case definitions (left) and the heat map of the Bonferroni-corrected *p*-values for pairwise comparisons with the Kruskal–Wallis test comparing the significance of differences among the various case definitions (right). In the left panels, each dot represents one participant (yellow = periodontal health, light green = gingivitis, dark green = stage I periodontitis, light blue = stage II periodontitis, dark blue = stage III periodontitis and black = stage IV periodontitis); the horizontal bars in the scatter plots display the medians. In the right panels, darker blue values show highly significant differences in concentrations between the different case definitions. The index scale of the significance (*p*-value) from dark blue to red is shown on the right of each heatmap plot. The upper row displays results for aMMP-8 concentrations (a), while the lower one (b) displays values corrected by the number of teeth that are present (aMMP-8/NTP). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (Bonferroni-corrected Kruskal–Wallis test). Stage I, stage I periodontitis; Stage II, stage II periodontitis; Stage III, stage III periodontitis; Stage IV, stage IV periodontitis.

3.3 | Associations of the Quantitative aMMP-8 Results With Periodontal Clinical Parameters

Since aMMP-8 POC-ORT detects the total concentration of aMMP-8 from an oral rinse sample representing the entire mouth, the relationship between aMMP-8 levels, aMMP-8/NTP and the number or proportion of diseased sites in periodontitis patients warrants further clarification. As shown in Figure 3, for all participants the aMMP-8 concentration showed a low to moderate positive correlation with the number and proportion of sites exhibiting deep PPD, interdental CAL, BOP and deep bleeding pockets.

In both studies, the correlation between aMMP-8 levels and the number or proportion of sites with interdental CAL was relatively weak, suggesting that aMMP-8 concentration was more closely associated with sites exhibiting active periodontal inflammation. In the Hong Kong study, the correlation between aMMP-8 levels and clinical parameters was consistent across most stages, with the exception of stage I periodontitis which showed no statistically significant correlation. In contrast, the Shanghai study revealed higher correlation coefficients for stages II and IV compared to other stages.

To further explore the relationship between aMMP-8, aMMP-8/NTP and clinical parameters, we performed simple and multiple linear regression analyses (Table 2). The results revealed that the number of sites exhibiting deep PPD, BOP and deep bleeding pockets significantly influenced both aMMP-8 and aMMP-8/NTP levels. Among these factors, the number of sites with PPD $\geq$ 5 mm and BOP had the most substantial effect on both aMMP-8 and aMMP-8/NTP values. The adjusted regression coefficients (1.298 for Hong Kong and 0.705 for Shanghai) were statistically significant ($p < 0.001$), indicating that for each additional site with PPD $\geq$ 5 mm and BOP, the aMMP-8 value increased by 1.298 and 0.705 ng/mL in the Hong Kong and Shanghai cohorts, respectively.

The two additional data processing methods mentioned above were also applied to the correlation and regression analysis, and

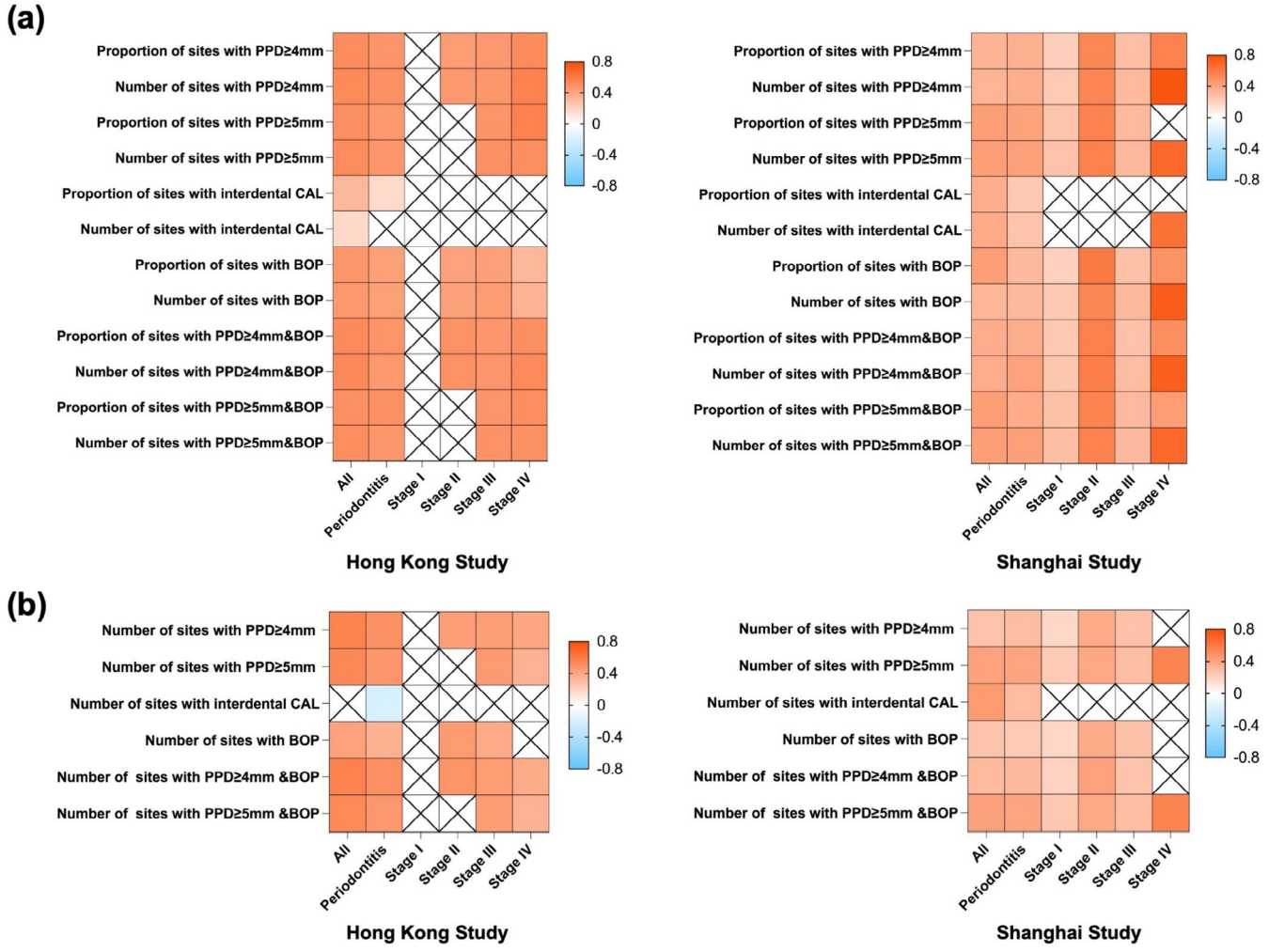

FIGURE 3 | Spearman's rank correlations heatmaps between aMMP-8 concentrations and periodontal clinical parameters for different case diagnoses. The different panels show the heatmaps of Spearman's correlation coefficient (ρ) between the quantitative aMMP8, aMMP8/NTP levels and periodontal clinical parameters in the Hong Kong (left column) and Shanghai (right column) studies. The reference index is shown on the right of each diagram with values between −0.8 in blue (negative correlation) and 0.8 in dark red (positive correlation). (a) aMMP8 concentrations. (b) aMMP8/NTP levels. Heatmaps show the values of ρ. × indicates no statistical significance ($p > 0.05$, Spearman's ρ); all other comparisons are statistically significant. Stage I, stage I periodontitis; Stage II, stage II periodontitis; Stage III, stage III periodontitis; Stage IV, stage IV periodontitis.

TABLE 2 | Simple and multiple linear regression analyses for the association of the aMMP-8 POC-ORT with periodontal clinical parameters.

	Hong Kong study						Shanghai study					
	Crude			Adjusted[a]			Crude			Adjusted[a]		
aMMP-8 value	R^2	*B*	95% CI	R^2	*B*	95% CI	R^2	*B*	95% CI	R^2	*B*	95% CI
Number of sites with interdental CAL	0.021	0.214	0.068–0.359	/	/	/	0.185	0.297	0.235–0.359	0.196	0.376	0.279–0.473
Number of sites with BOP	0.168	0.39	0.302–0.477	0.205	0.37	0.283–0.457	0.123	0.261	0.191–0.330	0.209	0.275	0.208–0.342
Number of sites with PPD ≥ 5 mm	0.198	1.289	1.028–1.549	0.238	1.272	1.008–1.536	0.275	0.732	0.230–0.319	0.293	0.681	0.559–0.804
Number of sites with PPD ≥ 5 mm and BOP	0.201	1.327	1.060–1.593	0.238	1.298	1.029–1.567	0.277	0.757	0.635–0.879	0.297	0.705	0.579–0.831
Number of sites with PPD ≥ 4 mm	0.215	0.75	0.606–0.894	0.252	0.746	0.598–0.894	0.175	0.386	0.303–0.47	0.25	0.39	0.309–0.47
Number of sites with PPD ≥ 4 mm and BOP	0.215	0.776	0.627–0.925	0.249	0.761	0.609–0.913	0.209	0.437	0.352–0.522	0.275	0.43	0.348–0.513
	Hong Kong study						Shanghai study					
	Crude			Adjusted[a]			Crude			Adjusted[a]		
aMMP-8/NTP value	R^2	*B*	95% CI	R^2	*B*	95% CI	R^2	*B*	95% CI	R^2	*B*	95% CI
Number of sites with interdental CAL	/	/	/	/	/	/	0.198	0.012	0.010–0.014	0.199	0.012	0.009–0.016
Number of sites with BOP	0.079	0.013	0.008–0.017	0.135	0.011	0.007–0.016	0.088	0.009	0.006–0.011	0.214	0.009	0.007–0.012
Number of sites with PPD ≥ 5 mm	0.162	0.056	0.043–0.068	0.21	0.053	0.04–0.066	0.27	0.028	0.009–0.013	0.313	0.025	0.021–0.03
Number of sites with BOP and PPD ≥ 5 mm	0.162	0.057	0.044–0.070	0.209	0.053	0.04–0.067	0.272	0.029	0.024–0.034	0.315	0.026	0.021–0.031
Number of sites with PPD ≥ 4 mm	0.165	0.031	0.024–0.038	0.209	0.03	0.022–0.037	0.152	0.014	0.011–0.017	0.266	0.014	0.011–0.017
Number of sites with BOP and PPD ≥ 4 mm	0.161	0.032	0.025–0.039	0.204	0.03	0.022–0.037	0.184	0.016	0.013–0.019	0.289	0.016	0.013–0.019

Note: The results are presented as statistically significant (p-value <0.05); otherwise, they are denoted as '/'.

Abbreviations: 95% CI, 95% confidence interval; aMMP-8/NTP, total aMMP-8 concentration divided by the number of teeth present; BOP, bleeding on probing; CAL, clinical attachment loss; PPD, probing pocket depth.

[a]Adjusted for age, smoking and systemic condition.

the results (shown in Tables S3 and S4) were consistent with those shown in Figure 3 and Table 2.

3.4 | Associations of the Dichotomised aMMP-8 Results With Periodontal Clinical Parameters

To further clarify the relationship between dichotomised aMMP-8 results and periodontal clinical parameters, logistic regression analyses were performed, as shown in Figure 4. In both studies, the number of sites with deep PPD, interdental CAL, BOP and deep bleeding pockets significantly affected the likelihood of obtaining a positive result. Notably, the number of sites with PPD ≥ 5 mm and BOP had the most substantial impact. In the Hong Kong study, the adjusted odds ratio (OR) was 1.093 (95% CI: 1.064–1.129), while in the Shanghai study it was 1.077 (95% CI: 1.052–1.105). The adjusted OR for the number of sites with interdental CAL was the lowest, with values of 1.013 (95% CI: 1.004–1.023) in the Hong Kong study and 1.029 (95% CI: 1.018–1.041) in the Shanghai study.

3.5 | Clinical Features of Misclassified Cases

Based on the findings outlined above, we hypothesise that the high FN rate of aMMP-8 POC-ORT is due to the limited number of inflammatory sites in some cases, which results in a lower overall aMMP-8 concentration and contributes to missed diagnoses. To investigate this, we compared the clinical presentation of FN and true-positive (TP) cases. As shown in Figure 5, the number of sites with deep PPD, BOP and deep bleeding pockets—but not interdental CAL—in the FN cases was significantly lower than that in the TP cases. The extent of inflammation and inflamed pockets therefore was more localised in FN cases. The analysis of false-positive (FP) and true-negative (TN) cases (Figure S1) was characterised by fewer misclassified cases, which did not allow a detailed analysis by periodontitis stage. TN cases had fewer pockets, BOP and bleeding pockets than FP cases in the Hong Kong database but not in the Shanghai one. Interestingly, misclassification did not appear to be associated with the number of sites with detectable interdental CAL.

4 | Discussion

This study systematically investigated the key determinants influencing the diagnostic accuracy of aMMP-8 POC-ORT in oral-rinse-based periodontitis screening. Our findings reveal two interrelated sources of misclassification: (1) the limitations in collecting biomarkers with oral rinses, which are influenced by the number of inflamed periodontal lesions present in a subject, and (2) the analytical constraints stemming from the test's analytical sensitivity thresholds and cut-off selection.

The aMMP-8 test results from oral rinses showed a low to moderate correlation with the number and proportion of periodontal pockets and BOP. Notably, the number of sites with PPD ≥ 5 mm and BOP significantly impacted aMMP-8 POC-ORT results, whether measured by dichotomised outcomes, aMMP-8 concentrations or aMMP-8/NTP values. The results are consistent with the notion that oral rinse samples represent the pooling of GCF biomarkers from the affected sites of the dentition and, thus, that more widespread lesions lead to higher detectable concentrations. Mechanistically, this is supported by larger GCF volumes and flow rates in sites with deeper probing depths and inflammation (Ozkavaf et al. 2000; Griffiths et al. 1992) and by the high local concentrations of valid biomarkers, including aMMP-8, in the periodontal lesions (Arias-Bujanda et al. 2019). It also agrees with recent data showing that MMP-8 concentrations predict progressing periodontitis at multiple sites (Teles et al. 2024). A consistent pattern emerged from the analysis of misclassifications: FN cases exhibited significantly fewer pockets and BOP or bleeding pockets than TP cases. This trend was observed across case definitions and in both studies.

Prior investigations of MMP-8/clinical parameter correlations have predominantly (1) compared GCF MMP-8 levels with single-site clinical metrics versus oral fluid MMP-8 with full-mouth averages (Gupta et al. 2015; Kraft-Neumärker et al. 2012; Lorenz et al. 2017); (2) assessed diagnostic performance using pre-defined thresholds (e.g., ≥ 2 sites with PD ≥ 4 mm) (Heikkinen et al. 2016; Nwhator et al. 2014); (3) analysed limited parameters (e.g., deep pockets/BOP counts) without staging adjustments (Gursoy et al. 2013; Heikkinen et al. 2023); or (4) reported basic correlations between aMMP-8 and diseased sites without regression/staging analyses (Deng et al. 2021, 2022). This is the first study to investigate the relationship between the high FN rate of the aMMP-8 POC-ORT and the number/proportion of diseased sites. FN cases showed significantly fewer inflammatory sites than TP cases ($p < 0.01$). In stage IV periodontitis, despite high inflammation rates at the individual sites, extensive tooth loss reduced the total number of affected sites, resulting in aMMP-8 concentrations below the detection threshold (10 ng/mL). This observation aligns with challenges posed by the 2018 periodontal

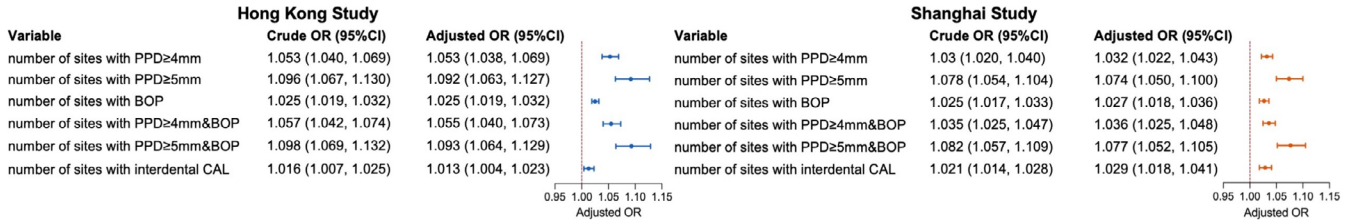

FIGURE 4 | Logistic regression analyses associating aMMP-8 positive tests with periodontal clinical parameters. Odds ratios show the likelihood of a positive aMMP-8 test result (with the threshold set at 20 ng/mL) associated with an increase in one clinical lesion (number of pockets, PPD or bleeding on probing, BOP). The adjusted OR was adjusted for age, smoking and systemic condition. All the results are statistically significant (p-value < 0.05). OR, odds ratio; 95% CI, 95% confidence interval.

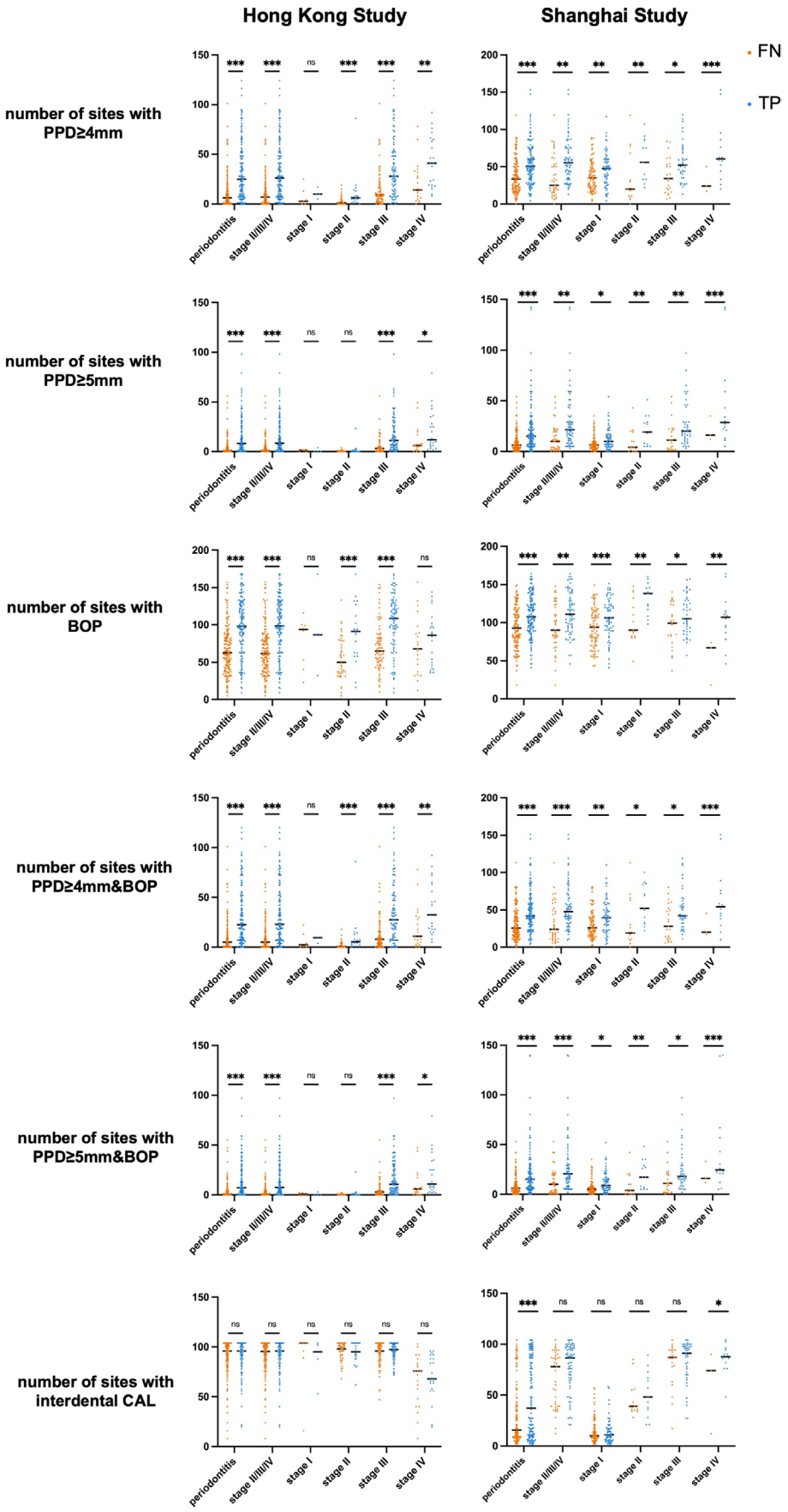

FIGURE 5 | Legend on next page.

FIGURE 5 | Comparison of the number of periodontal lesions among false-negative and true-positive test results. The figure compares the number of diseased sites between false-negative (FN) and true-positive (TP) cases for each periodontitis stage diagnosis in the Hong Kong (left column) and Shanghai (right column) studies. FN cases are shown in orange and TP cases are shown in light blue for each case diagnosis. Each dot represents one case, and the horizontal line shows the median number of sites. Comparisons between FN and TP were performed with the Mann–Whitney *U* test. Periodontitis comprises all cases with periodontitis; Stage I, stage I periodontitis; Stage II, stage II periodontitis; Stage III, stage III periodontitis; Stage IV, stage IV periodontitis; stage II/III/IV periodontitis comprises all cases. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns, not significant (Mann–Whitney *U* test).

classification system, where 'extent' assessment requires stage-specific interpretation (Sanz et al. 2020)—higher disease stages paradoxically correlate with fewer qualifying lesions, exacerbating sensitivity limitations in oral-rinse-based detection.

Although our study found that aMMP-8/NTP had a slightly higher AUC than aMMP-8 in diagnosing severe periodontitis, and aMMP-8/NTP showed differences across a broader range of periodontal status, correcting aMMP-8 concentrations by NTP offers only limited improvement to the overall diagnostic performance. It does not address the key limitations of the test.

Another noteworthy finding in the Hong Kong study is that the correlation between sites with interdental CAL and aMMP-8 value was significantly lower compared to other clinical parameters. This might suggest that aMMP-8 may not be a suitable test for distinguishing between different stages of periodontitis. However, the stronger correlation between aMMP-8 and inflammation-related parameters such as BOP and PPD supports its potential use for detecting active inflammation sites, probably making it a valuable tool for surveillance and treatment monitoring for periodontitis, combined with the findings of other proof-of-principle studies (Heikkinen et al. 2023; Leppilahti et al. 2011; Sorsa et al. 2016).

It is also important to emphasise that when discussing the aMMP-8 test, the fluid source must be specified. This is because the diagnostic performance of aMMP-8 in GCF, saliva and oral rinse can vary depending on the oral fluid used and should not be conflated (Arias-Bujanda et al. 2019, 2020; Keles Yucel et al. 2020). In this study, all conclusions regarding aMMP-8 POC-ORT are based on oral rinse samples and are intended for periodontal disease screening.

Comparative analyses of diagnostic thresholds reveal critical trade-offs in aMMP-8 testing efficacy. Deng et al. (2021) showed that increasing the threshold from 10 to 20 ng/mL universally reduced sensitivity while improving specificity across periodontitis classifications. This aligns with Keskin et al.'s (2023) recommendation of 20 ng/mL as an optimal cut-off for monitoring advanced-stage (III/IV-grade C) periodontitis treatment. A 2024 meta-analysis by Wei et al. (2024) quantified this threshold-dependent performance, reporting pooled sensitivity/specificity of 0.53 (95% CI: 0.21–0.84)/0.94 (0.83–0.99) at 20 ng/mL versus 0.55 (0.18–0.88)/0.81 (0.48–0.97) at 10 ng/mL. Our data further highlight a population-specific optimal cut-off: Youden index identified 24 ng/mL (Hong Kong study) and 13 ng/mL (Shanghai study) as ideal thresholds. Alarmingly, the conventional 20 ng/mL threshold failed to detect > 50% of early-stage (I/II) cases, strongly advocating for reduced cut-offs (< 20 ng/mL) in screening protocols. However, the current aMMP-8 POCT system is unable to detect concentrations below 10 ng/mL. This limitation reduces the test's sensitivity for cases with fewer affected sites, restricts the possibility of exploring more optimal thresholds and complicates the analysis of quantitative aMMP-8 values. Therefore, further technological or sampling improvements may be necessary to detect lower concentrations of aMMP-8 in the POCT system and identify the optimal cut-off value. In this respect, it is essential to emphasise that aMMP-8 in oral rinses or saliva originates in the GCF. The number of teeth, their periodontal health status and the duration of sampling (oral rinse) determine the quantity of aMMP-8 cleared from the gingival crevices into the oral cavity.

5 | Conclusion

While aMMP-8 POC-ORT shows promise for monitoring periodontal inflammatory status, its current implementation as a stand-alone screening tool faces biological and technical constraints. The number of periodontal lesions in the dentition appears to be a key determinant of ora- rinse-based test results, leading to high FN rates in localised periodontitis. The diagnostic accuracy could be substantially improved through threshold and sampling optimisation, biomarker normalisation and technological advancements in low-concentration detection. These adaptations are worthy to be contextualised within refined periodontal classification systems to ensure clinically meaningful implementation for periodontal health care.

Author Contributions

M.S.T. conceived this work, wrote the study protocols and wrote the first draft of the report. L.J. and M.S.T. designed the original studies, secured the funding and interpreted the data. M.S.T. and Y.L. supervised the work. M.B., X.Y., Y.L., Y.Y., H.L. and G.P. analysed the data. All authors contributed to reviewing the draft and approved the final version.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available from the investigators for scientific collaboration upon reasonable request.

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