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Utilizing Oral Neutrophil Counts as an Indicator of Oral Inflammation Associated With Periodontal Disease: A Blinded Multicentre Study

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ABSTRACT

Background: Periodontal diseases are chronic inflammatory conditions that require early screening for effective long-term management. Oral neutrophil counts (ONCs) correlate with periodontal inflammation. This study investigates a point-of-care test using a neutrophil enzyme activity (NEA) colorimetric strip for measuring periodontal inflammation.

Methods: This prospective study had two phases. Phase 1 validated the relationship between ONCs and periodontal inflammation with 90 participants. Phase 2 examined the test's applicability in a real-world setting through a multicentre clinical trial with 375 participants at four sites. ONCs were quantified in oral rinses using laboratory-based methods, and the NEA strip was used for ONC stratification. Clinical measures included bleeding on probing (BoP), probing depth (PD) and clinical attachment loss (CAL).

Results: ONCs were significantly elevated in patients with Grade B periodontitis and deep periodontal pockets (PD ≥ 5 mm, CAL ≥ 5 mm). The NEA strip accurately classified patients into high or low ONC categories, showing 80% sensitivity, 82.5% specificity and an AUC of 0.89. It also assessed the effectiveness of periodontal therapy in reducing ONC and inflammation. The test was user-friendly, with no reported discomfort among patients.

Conclusion: The NEA strip is a user-friendly and rapid screening tool for detecting high ONCs associated with periodontal inflammation and for evaluating the effectiveness of periodontal therapy.

1 | Introduction

Periodontal diseases are universally prevalent chronic inflammatory conditions linked to systemic diseases such as cardiovascular, cardiometabolic and autoimmune disorders (Winning and Linden 2015; Zemedikun et al. 2021). The subclinical symptoms of periodontal diseases, along with their episodic nature of progression (Loos and Van Dyke 2020), highlight the need for early screening tools that allow long-term monitoring of periodontal health. Inflammatory periodontal diseases are driven predominantly by oral bacteria and, when left untreated, can lead to the development of progressive destruction of tooth-supporting structures and, ultimately, loss of teeth (Kinane, Stathopoulou, and Papapanou 2017). Conventional methods for

detecting periodontal disease involve the use of a periodontal probe inserted into the gingival sulcus to measure periodontal probing depths (PDs), bleeding on probing (BoP) and clinical attachment loss (CAL). While PD and CAL reflect the periodontium's condition at the time of examination, they cannot detect ongoing tissue destruction or reliably predict future periodontal breakdown.

In healthy oral tissues, there is a balance between oral bacteria and the influx of innate immune cells, predominantly oral polymorphonuclear neutrophils (oPMNs) (Fine et al. 2020). When periodontopathogenic bacteria begin to accumulate and initiate dysbiosis within the oral microbiome, this balance is disrupted. This leads to an increased number of oPMNs being trafficked to the gingiva to clear the bacteria and prevent tissue invasion (Scott and Krauss 2012). This microbial–immune interaction triggers a cascade of pro-inflammatory mediators as well as accumulation of extracellular degradative enzymes. At high local levels, these mediators lead to the breakdown of the soft and subsequently hard tissues of the periodontium, resulting in periodontal pockets formed as a consequence of this degradation. These characteristics are considered a hallmark of periodontal diseases, particularly periodontitis (Ejeil et al. 2003; Sapna, Gokul, and Bagri-Manjrekar 2014). To treat this, subgingival plaque and calculus removal is generally effective in pockets <5 mm deep, yet successful removal becomes less effective in deeper pockets (Waerhaug 1978). When all plaque and calculus is removed, the dento-epithelial junction starts to normalize and then readapts to the 'cleaner' root surface.

Accumulating evidence has found an association between the number of oPMNs and the presence of periodontal inflammatory diseases (Khoury et al. 2020). Based on studies carried out previously in our laboratory and others, it is evident that the number of oPMNs, as quantified in oral rinses, represents a reliable measure of periodontal inflammation (Bender, Thang, and Glogauer 2006; Landzberg et al. 2015). The methodology developed permits non-invasive quantification of oPMNs. The correlation between elevated oPMN counts and periodontal disease severity highlights the potential of using oPMN counts as an indirect biomarker for monitoring periodontal health and inflammation while providing valuable insights into the innate immune-cell dynamics of oral inflammation.

A growing body of evidence supports the notion that the oral neutrophil count (ONC) corresponds not only to the presence of active periodontal inflammation but also to the severity of inflammation, underlining its significance as an informative marker for recruitment of active oPMNs, which can be related to downstream destruction of periodontal tissues (Landzberg et al. 2015).

Thus, this study provides a promising avenue for the development of novel dental screening tools. The purpose of this screening tool is not to diagnose periodontal disease, as oPMN counts do not necessarily reflect the history of periodontal disease, but to enrich individuals in whom further investigations can be used more selectively and to assist making decisions insofar as interventional treatments are concerned.

Despite the promise of using the ONC as a screening tool for the degree of oral inflammation present in the mouth, the quantification of oPMNs is usually performed in a laboratory setting, requiring the use of various types of equipment, ranging from simple laboratory equipment (optical microscope and haemocytometer) to more complex equipment (cytofluorometers). This complexity makes it challenging to use ONC to measure periodontal inflammation in dental settings on a routine basis.

Recognizing the potential significance of ONCs in assessing gingival inflammation, our study sought to assess the effectiveness and safety of using a point-of-care test (POCT) device that measures neutrophil enzyme activity by employing a colorimetric strip test (strip test; OSI, Montreal, Canada). This strip uses a colourimetric reaction to detect PMN-associated myeloperoxidase activity in an oral rinse sample, where the depth of colour change corresponds to the number of PMNs present. As a POCT device, it allows real-time chairside screening for periodontal inflammation and requires less than 4 min of operator time. Its importance is amplified by the growing body of evidence highlighting the connections between periodontal and systemic health. The simplicity and speed of this method could extend its use to non-dental healthcare professionals, including physicians and pharmacists, facilitating early detection and intervention of periodontal inflammation.

This multicentre, prospective study was conducted on two phases. Phase 1 was to investigate the relationship between ONC and the periodontal status and show that the POCT accurately reflects ONCs as compared to validated laboratory testing. Meanwhile, Phase 2 aimed to evaluate the efficacy and safety of this POCT as an aid to the detection of oral inflammation (characterized by the standard clinical measures noted above) in real-world clinical practice.

2 | Methods

2.1 | Study Design

This multicentre, prospective study aimed to assess the effectiveness and safety of the NEA strip test for measuring ONC as a measure of periodontal inflammation. The study was conducted in two phases: a pilot study with 90 participants performed at the University of Toronto from December 2022 to June 2023 (patients who had not had regular care and were not aware of dental problems), followed by a large-scale study with 375 participants at four clinical sites, two in the United States and two in Canada (patients scheduled for their regular dental hygiene visits) from May 2023 to June 2023. Both studies received necessary approvals (REB00039888, NCT05886855).

The participants met the following inclusion criteria: (1) older than 18 years, (2) fluent in English and (3) no active oral lesions. Medically compromised patients were excluded if probing could pose a risk or interfere with the accuracy of the results. These conditions include, but are not limited to (1) patients with uncontrolled medical conditions or are neutropenic who can have an increased risk of infection following probing (Zimmermann et al. 2015) and (2) patients on certain medications, such as anticoagulants, who may experience more bleeding upon dental probing (Royzman et al. 2004). The study was explained to each participant, and informed consent was obtained from all participants.

2.2 | Oral Rinse Samples and NEA Strip Testing

Oral rinse sample collection was performed as described previously (Forster et al. 2012; Aboodi et al. 2015). Briefly, subjects were asked to pre-rinse their mouths with tap water for 15 s to eliminate leukocytes (including oPMNs) and debris. Following the pre-rinse, patients were instructed to wait for 2 min to allow the influx of PMNs into the oral cavity (such influx being indicative of active inflammation depending on the level that is eventually measured). Subsequently, a 30-s rinse was performed using 10 mL of USP-grade water. The participants were then asked to expectorate their oral rinses into a reaction cup. The test strip was then dipped in the rinse for 1 s, allowing enzymes to catalyse the reaction strip. The final reading of the NEA test strip colour was performed after 60 s by comparing it with a proprietary colour chart (Figure S1). The intensity of the purple colour should correlate with oPMN counts (and be confirmable and comparable to data obtained by lab testing). The colour was graded as being representative of no oPMN (negative), or low, medium or high level of ONC. The gradations of negative and low were concatenated so as to represent a 'negative reading', while readings of medium and high ONC were concatenated so as to represent simply a 'positive reading'. This manoeuvre allowed the performance of bivariate data analyses when needed. For the multicentre cohort, 19 operators performed this test at our four sites (at least three operators per site). After using the NEA strip test, the comfort of the participants was recorded (yes/no) on the case report form. In addition, operators were asked to complete a questionnaire regarding the ease of using the NEA test. In total, 17 operators sent the back the completed questionnaires for assessment.

2.3 | Clinical Measures of Inflammation

After the oral rinse samples were taken, a full-mouth periodontal examination was conducted, which included BoP, PD and CAL at six sites per tooth using a Michigan O Probe. Following the guidelines set forth by the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, participants were stratified based on the percentage of sites with BoP. This classification distinguished between localized gingivitis (10%–30% of sites with BoP) and generalized gingivitis (over 30% of sites with BoP), reflecting the extent of gingival inflammation present. However, as this group of patients presented to the University of Toronto seeking treatment for ongoing dental disease, none of the patients presented with less than 10% BoP, meaning that none was healthy from a periodontal perspective, and so a 'healthy' group could not be used for comparison. This detailed stratification is crucial, as it aligns the clinical assessments performed in this investigation with the latest consensus, allowing for a nuanced understanding of periodontal health which is vital for the precise evaluation of the POCT device's effectiveness. A total of 69 patients were re-evaluated 2 weeks after receiving non-surgical periodontal treatment (scaling and root planing).

A clinical examination that included only BoP was conducted for the second phase of the study, which was performed on the multicentre cohort. Once again, the consensus alluded to above was used in order to stratify participants. Unlike the first cohort, these participants were scheduled for their regular examination and not particularly seeking treatment for ongoing disease. This allowed us to have a healthy group for comparison. No re-evaluation was conducted for the participants of this cohort.

2.4 | oPMN Quantification and ONC Determination

For laboratory testing, the oral rinse samples were fixed immediately by pouring them into a 15-mL tube containing 2 mL of phosphate buffered saline (PBS) 10× and paraformaldehyde. The tubes were sent to a laboratory for quantification of oPMN levels. The oPMNs in each oral rinse specimen were counted using a validated method based on cell fixation and subsequent staining with acridine orange (Sigma Chemical, Burlington, ON, Canada). Acridine orange is a fluorescent nucleic acid marker that allows technicians to distinguish oPMNs from other cells using a fluorescence microscope. The oral rinse sample (500 μL) was centrifuged at 6000 rpm for 5 min and resuspended in 100 μL of PBS. Acridine orange (1 μL) was then added to the sample, and a 10-μL aliquot of this suspension was evaluated using a haemocytometer under a fluorescence microscope (Leitz Orthoplan Microscope; Leitz, Wetzlar, Germany). Counts of oPMNs were performed visually using the haemocytometer. The technician performing the counting was unaware of the results obtained using the NEA test as well as any clinical measures (e.g., BoP). Based on previous studies, a threshold of 5.0×10^4 oPMNs/mL was used to distinguish between participants with high and low ONC, with having $\geq 5.0 \times 10^4$ oPMNs/mL as high ONC, and having $< 5.0 \times 10^4$ oPMNs/mL as low (Hans, Goswamy, and Hans 2020; Rijkschroeff et al. 2016).

2.5 | Statistical Analysis

Correlations were determined using Pearson's correlation coefficients. Continuous variables were compared using Student's *t*-test or one-way ANOVA variance, as appropriate. Fisher's exact test was used to compare differences in categorical variables (dichotomized variables). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios of the ability of the test strip to diagnose high ONC were calculated. A receiver operating characteristic (ROC) curve was used to assess the diagnostic capability of the NEA test for diagnosing high ONC levels and the area under the curve (AUC). Analyses and plots were generated using GraphPad Prism version 9.3.

3 | Results

3.1 | Patient Characteristics for Phase 1

In total, 90 participants were included in the first cohort (Table 1). All 90 participants who were recruited in this phase of the study sought treatment at the Faculty of Dentistry at the University of Toronto as part of a cohort who had no access to dental care for financial reasons.

TABLE 1 | Clinical characteristics of the patients enrolled in Phase 1 of the study ($n = 90$).

	N	%
Total no. of subjects	90	
Age		
Mean	37.9	
Range	(17–69)	
Gender		
Female	42	47
Male	48	53
Health conditions		
Implants	1	1
Smoking	21	23
Diabetes	1	1

3.2 | Correlation Between ONC and Periodontal Clinical Parameters

First, we examined the relationship between ONCs and clinical measures of periodontal disease in the first cohort. A moderate correlation was found between ONCs and BoP percentage within the patients ($r = 0.51$, $p < 0.001$) (Figure 1a). All patients who sought treatment presented with gingival inflammation, resulting in the lack of a healthy control group in the initial cohort. A total of 13 participants were in the $10\% < \text{BoP} < 30\%$ group and 77 in the $\text{BoP} > 30\%$ group, with oPMN counts demonstrating a significant difference between the two groups with a mean of 6.5×10^4 and 13.1×10^4, respectively (Figure 1b). Additionally, when assessing oPMN counts distributed across the participants' periodontal stage diagnosis, means of 11.6×10^4, 14.7×10^4 and 36.9×10^4 oPMN/mL were observed representing Stage 1, Stage 2 and Stage 3 periodontitis, respectively (Figure 1c). Similarly, there was a significant increase in oPMN counts associated with Grade B periodontitis compared to Grade A.

A threshold of 5.0×10^4 oPMN/mL was used to stratify subjects into groups characterized by one or more sites with $\text{PD} \geq 5\,\text{mm}$ or $\text{CAL} \geq 5\,\text{mm}$ (Figure S2). For PD, the test demonstrated high sensitivity of 91% and a high NPV of 88%, making it effective for ruling out having periodontal pockets deeper than 5 mm when the result is negative. However, its low specificity (38%) and moderate PPV (48%) indicated that there was a significant propensity for finding false positive test results, suggesting that positive results should be confirmed with further clinical examination. The same was noted with CAL, for which test sensitivity was 94% and the NPV was 92% (high), but with a low specificity of 38% and a moderate PPV of 45%.

3.3 | Correlation Between the NEA Strip and ONC

After verifying the correlation of ONC with periodontal disease grade, BoP and having >5 mm pockets, we wanted to examine the accuracy of the POCT in classifying patients into four categories (negative, low, medium and high) reflecting these counts. Indeed, samples classified as healthy with a negative or low strip test result displayed a mean ONC $< 5.0 \times 10^4$ oPMN/mL oral rinse (Figure 2a). Meanwhile, samples classified as medium or high corresponded to measures of ONC of $> 5.0 \times 10^4$ oPMN/mL in the oral rinse. When assessing the accuracy of a negative (negative or low) or positive (medium or high) result of correctly assigning participants based on the 5.0×10^4 threshold, the NEA strip test displayed high sensitivity, specificity, PPV and NPV (Figure 2b).

3.4 | NEA Strip Results in Relation to the Patient's Periodontal Diagnosis

While ONC correlated with some measures of periodontal inflammation and the NEA Strip test reflected the ONC, we wanted to confirm how well the results obtained using the POCT aligned with clinical parameters. There was a significant difference in BoP found between those in whom a positive POCT result was found (i.e., medium + high) and those in whom a negative result (i.e., negative + low) was found (Figure 3a). The periodontal diagnosis of the participants was then distributed across the different NEA strip test results. It was found that 83.4% of patients with Stage 2 periodontitis and 100% of those with Stage 3 had positive (i.e., medium + high) test results using the NEA system.

In categorizing participants with $\text{PD} \geq 5\,\text{mm}$ at one or more sites, the NEA strip test had a sensitivity of 81%, specificity of 47%, PPV of 38% and NPV of 86% (Figure S3a). Similarly, for detecting $\text{CAL} \geq 5\,\text{mm}$ at one or more sites, the test displayed a sensitivity of 92%, specificity of 45%, PPV of 40% and NPV of 94%. This indicates that the test is effective in ruling out periodontal disease in the absence of the disease but has limitations in confirming diagnoses because of its moderate specificity and PPV. Thus, the test is more reliable for screening and excluding these periodontal conditions than for definitive diagnosis.

3.5 | NEA Test Results and ONC Changes at Follow-Up After Therapy

Patients were followed up with a full clinical assessment 2 weeks after periodontal treatment. Patients were categorized into a responder group (displaying a decline in BoP of at least 5%), a non-responder group (having less than 5% change in BoP) and progressors (displaying more than a 5% increase in BoP) (Figure 4a–c). ONC trends followed a similar pattern to BoP in the responder group, showing a significant reduction at follow-up compared to baseline. In contrast, no evident reduction was seen in the non-responder or the progressor groups (Figure 4d–f). When the NEA test results at follow-up were compared to baseline (Figure 4g), most responders showed a decrease in strip colour result (62%) or no change (38%).

3.6 | Phase 2 Participant Characteristics

In total, 375 participants were included in this study (Table 2). The participants were recruited from four outpatient dental

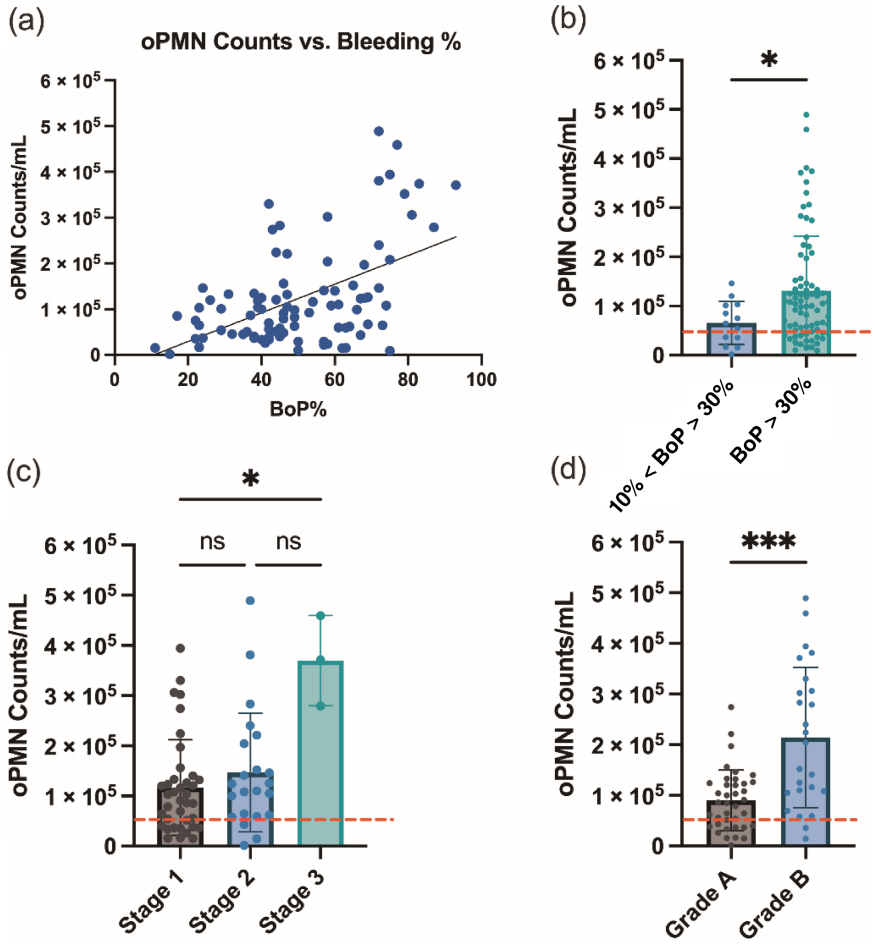
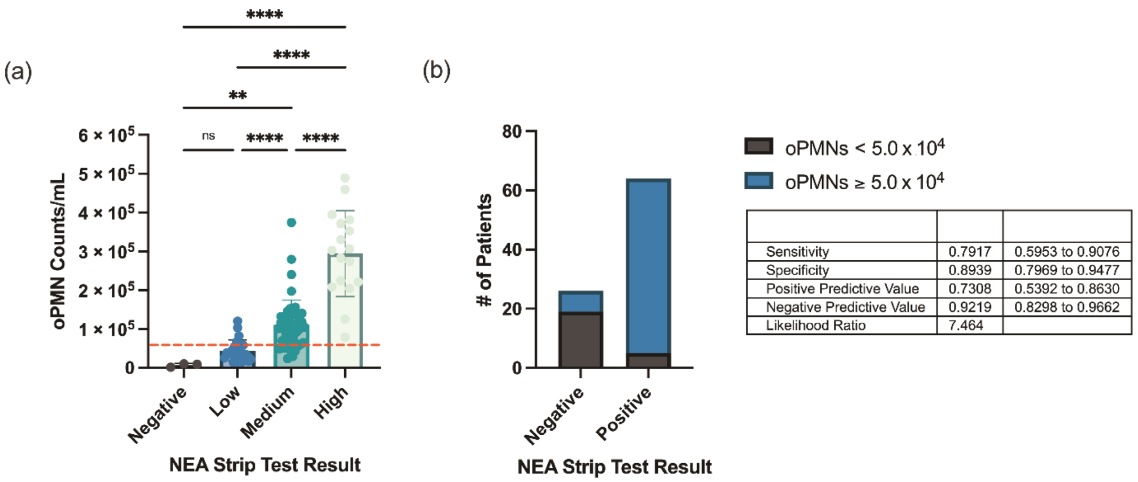


FIGURE 1 | ONC in relation to clinical parameters. (a) A correlation was made between ONC and the BoP% using the Pearson correlation coefficient. (b) Patients were categorized based on their BoP%, and the oPMN count of each group was compared: BoP < 10% (healthy), 10% < BoP < 30% (localized gingival inflammation) and BoP > 30% (generalized gingival inflammation). However, there was no healthy participants in this cohort. ONCs were compared across different (c) stages and (d) grades of periodontitis. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.0005$; ****$p \leq 0.0001$. The bars in the graph on the left represent mean values, and the vertical lines are the standard deviations.



Sensitivity	0.7917	0.5953 to 0.9076
Specificity	0.8939	0.7969 to 0.9477
Positive Predictive Value	0.7308	0.5392 to 0.8630
Negative Predictive Value	0.9219	0.8298 to 0.9662
Likelihood Ratio	7.464	

FIGURE 2 | The NEA strip test correlates to ONC. The OSI NEA test was used to stratify patients into (negative, low, medium, and high) based on the colorimetric reaction. (a) Plot of oPMN counts plotted according to the strip test classification with mean counts of 0.7×10^4, 4.4×10^4, 11.1×10^4 and 29.4×10^4. (b) Distribution of high and low ONC in the OSI NEA test (negative [negative and low], positive [medium and high]). *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.0005$; ****$p \leq 0.0001$. The bars in the graph on the left represent mean values, and the vertical lines are the standard deviations.

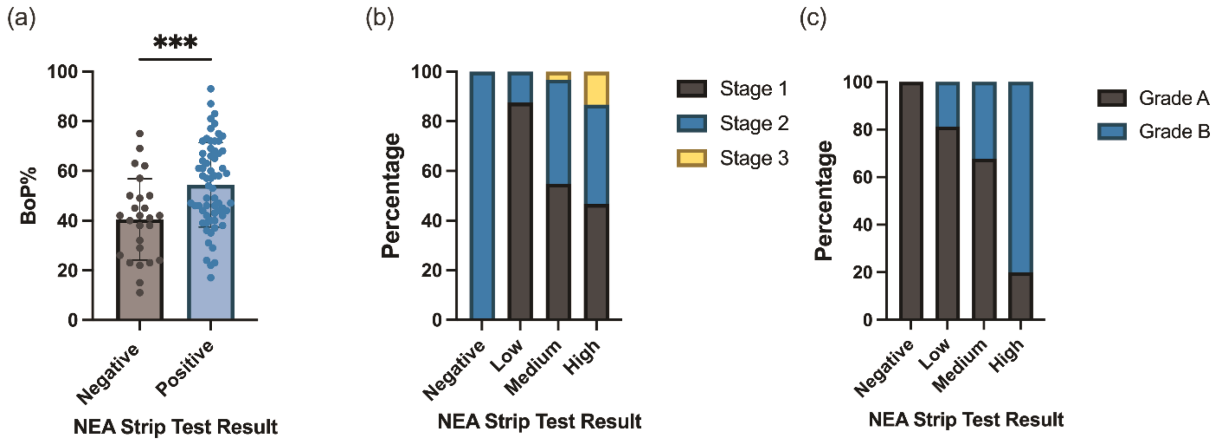


FIGURE 3 | The NEA strip test in relation to periodontal clinical parameters. (a) Comparing BoP% in patients with negative (negative and low) and positive (medium and high) test results. The distribution of different periodontitis (b) stages and (c) grades across the different NEA strip test results. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.0005$; ****$p \leq 0.0001$. The bars in the graph on the left represent mean values, and the vertical lines are the standard deviations.

clinics. All subjects were recruited from among the patients who visited their scheduled regular dental hygiene visits.

3.7 | Accuracy of the NEA Test in Measuring ONC and Reflecting BoP% in a Multicentre Setting

Similar to the first cohort, ONC showed a moderate correlation with BoP and corresponded to healthy, localized and generalized gingival inflammation (Figure S4). Hence, for the second phase of the study, the aim was to examine the applicability and accuracy of the NEA test in a dental clinic hygiene recall setting. Samples classified as healthy with a negative or low strip test result displayed a mean BoP of <10% and $<5.0\times10^4$ PMN/mL oral rinse (Figure 5a). Meanwhile, samples classified as medium or high corresponded to clinical measures indicating elevated gingival inflammation with a mean BoP > 10% and $>5.0\times10^4$ oPMN/mL oral rinse (Figure 5b).

3.8 | Diagnostic Performance of the NEA Test in a Hygiene Recall Setting

As the strip test reflects the ONC, we aimed to assess the clinical performance of the NEA test. The NEA test for high ONC correctly classified patients based on their ONC, with a sensitivity of 80%, specificity of 82.5%, PPV of 88%, NPV of 71% and a likelihood ratio of 4.537. Additionally, an ROC was generated for the NEA test as a predictor of high ONC, which displayed a high discriminatory ability with an AUC of 0.89 ($p < 0.0001$) (Figure 6a). Using the NEA test, 80.5% of the samples were correctly assigned their respective ONC statuses (Figure 6b). Similarly, when assessing the discriminatory ability of the test based on a negative or high result only, the test displayed a higher performance, with a sensitivity of 97%, specificity of 92%, PPV of 93%, NPV of 97% and a likelihood ratio of 12.01, with an AUC of 0.98 (Figure S5). Furthermore, the performance of the NEA test in the subgroups presented in Table 1 (demographics) was evaluated (Table S1). No significant differences were observed in the distribution of NEA scores among the subgroups. Meanwhile, the diagnostic performance of the 10% BOP threshold for detecting high ONC in the total 375 oral rinse samples was as follows: sensitivity (67%), specificity (59%), PPV (68%) and NPV (58%) with an AUC of (0.68) (Figure S6).

3.9 | NEA Test as a User-Friendly, Rapid Screening Tool

Next, we explored the applicability of the test in dental settings by evaluating both the patients' and operators' experiences while using the test. Patients reported that using the strip test did not cause any discomfort. This was also assessed by asking the operator questions regarding the comfort level of each participant. None of the 375 patients experienced discomfort while using the test strips.

The 19 operators were asked to complete a five-question survey to evaluate the ease of use (Table 3), of which 17 returned the completed questionnaire. All 17 operators strongly agreed that it was easy to apply the sample, interpret the results and follow the instructions associated with the test. Most operators (92.9%, 16/17) somewhat or strongly agreed that the instructions were easy to follow and that they did not require assistance to run the test. The operators reported use errors after performing the test for each participant. Use errors were defined as any issues encountered while collecting the specimens (requiring repetition of oral rinse, failure to collect the oral rinse) or any issue encountered with test reading (dipping time too long, reading time too short or too long, unexpected colour), and none was reported.

4 | Discussion

This study aimed to investigate the effectiveness and safety of POCT using a novel colourimetric strip based on measurement of neutrophil myeloperoxidase activity to detect ONC associated with inflammatory periodontal diseases. By integrating a multicentre prospective design that included a two-phase investigation, this study aimed at the detection of periodontal inflammation.

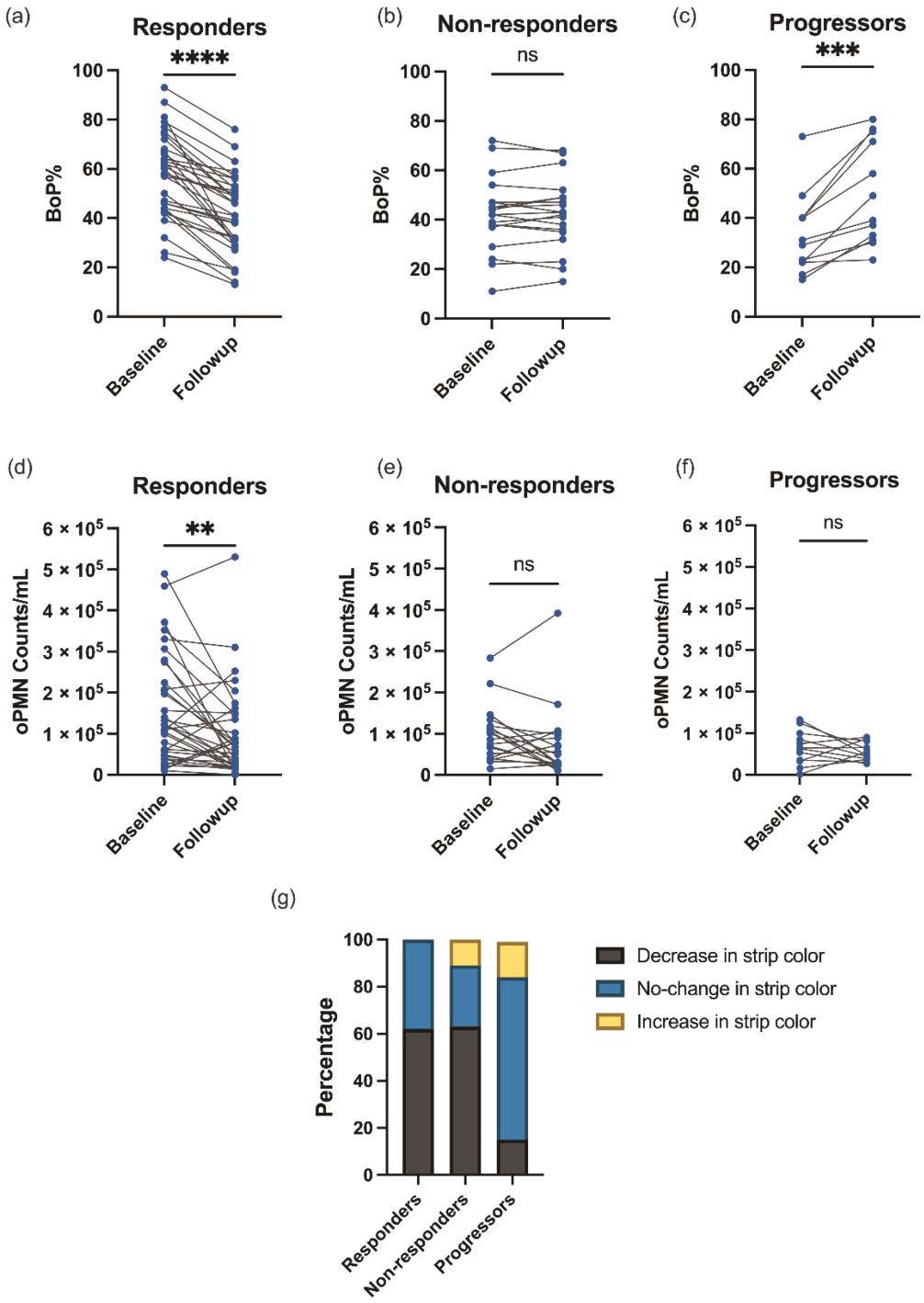


FIGURE 4 | Post-treatment response and NEA strip test change. Participants who had a follow-up examination ($n = 69$) were categorized into three groups based on the change in BoP%: (a) responder group (displaying a decline in BoP of >5%), (b) non-responder group (having <5% change in BoP) and (c) progressors (displaying >5% increase in BoP). ONC showed a decline in the responder group (d); meanwhile, ONC did not change in the non-responders (e) or the progressors (f). (g) Change in strip colour at follow-up vs. baseline was assessed across the different groups.

oPMNs are key components of active periodontal inflammation. The earliest description of PMN recruitment to the oral cavity was in the 1970s when the rate of orogranulocytic migration through gingival crevicular fluid was measured (Schiött and Löe 1970). A strong correlation was observed between the number of neutrophils migrating to the oral cavity and the degree of periodontal inflammation (Khoury et al. 2020). Different approaches have been used to quantify oPMNs, including saliva sampling, swabs and oral rinses. Our previous work focused on correlating ONC with some measures of inflammation, including BoP, PD and the gingival index (Landzberg et al. 2015). The current study reaffirms that ONCs are a reliable biomarker for active inflammatory activity including BoP%, grade of periodontitis and the presence of periodontal pockets deeper than 5 mm, which are risk factors for future tissue breakdown. ONC alone was not related to the various stages of periodontitis. This might be explained by the fact that staging of periodontal disease represents the 'history'

TABLE 2 | Clinical characteristics of the patients enrolled in Phase 2 of the study ($n = 375$).

	Site 1		Site 2		Site 3		Site 4		Total	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%
Total no. of subjects	199	53	21	6	99	26	56	15	375	100
Age										
Mean	60		40		59		67		60	
Range	(18–89)		(19–64)		(21–94)		(22–86)		(18–94)	
Gender										
Female	112	56	10	48	43	43	34	61	199	53
Male	87	44	10	48	56	57	22	39	175	47
NA	0	0	1	5	0	0	0	0	1	0.3
Ethnicity										
African American/ Canadian	7	4	9	43	0	0	0	0	16	4
Asian	12	6	5	24	2	2	0	0	19	5
Hispanic	1	1	3	14	0	0	0	0	4	1
Native	1	1	0	0	0	0	0	0	1	0.3
White	177	89	3	14	97	98	56	100	333	89
NA	1	1	1	5	0	0	0	0	2	1
Health conditions										
Implants	58	29	1	5	9	9	23	41	91	24
Smoking	13	7	1	5	8	8	6	11	28	7
Diabetes	13	7	0	0	7	7	5	9	25	7
Cancer(s)	6	3	0	0	2	2	0	0	8	2
Thyroid	0	0	0	0	2	2	4	7	6	2
High blood pressure	2	1	0	0	2	2	0	0	4	1
Other	3	2	0	0	6	6	5	9	14	4

Abbreviation: NA, information not available.

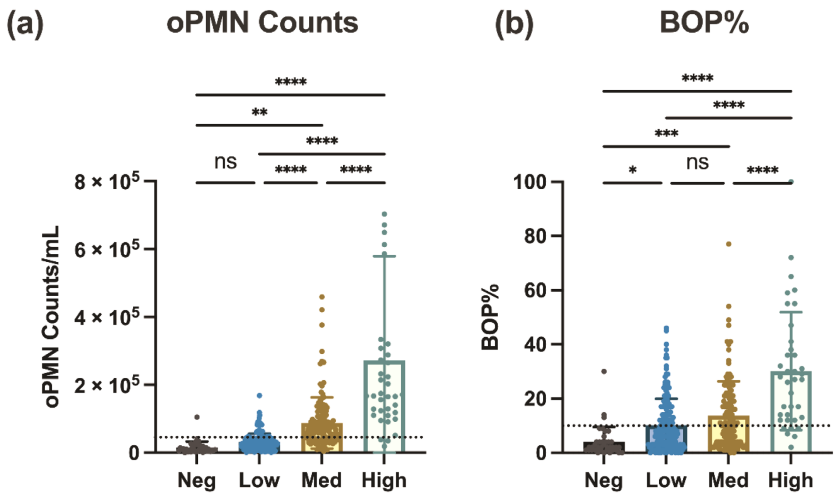


FIGURE 5 | oPMN level associated with high ONC. The OSI NEA test was used to stratify patients into (negative, low, medium and high) based on the colorimetric reaction. (a) Plot of oPMN counts plotted according to the strip test classification with mean counts of 1.5×10^4, 5.2×10^4, 31.8×10^4, 87.8×10^4 and 272.0×10^4. Similarly, the mean BoP% for each OSI NEA test category was recorded in (b), with a mean BoP of 4.0%, 10.1% and 30.2%.

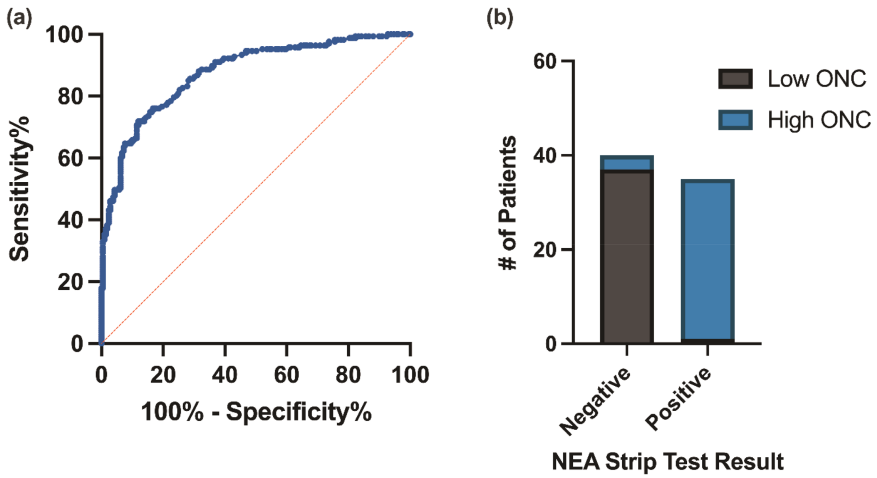


FIGURE 6 | OSI NEA test detection of high ONC. (a) ROC curve generated for the NEA test as a predictor of high ONC levels. (b) Distribution of high and low ONC in the NEA test (low [negative and low], high [medium and high]).

TABLE 3 | Results from the ease-of-test performance questionnaire recorded by the operators administrating the strip test.

Ease of use of NEA strip test (four sites; 17 operators)						
Question		Score (out of 17 operators)[a]				
No.	Description	1	2	3	4	5
1	The instructions were easy to follow.	0	0	0	0	17
2	It was easy to apply the sample correctly.	0	0	0	0	17
3	It was easy to see and understand the test results (e.g., appearance of the colour).	0	0	1	2	14
4	The instructions clearly present the warnings and precautions associated with the test.	0	0	0	1	16
5	I did not need help from someone the first time I ran the test.	0	0	0	2	15
	Total	0	0	1	5	79
	Rate	0%	0%	1.2%	5.9%	92.9%

[a]1 = *strongly disagree*; 2 = *somewhat disagree*; 3 = *neutral*; 4 = *somewhat agree*; 5 = *strongly agree*.

of disease activity and does not reflect the current status of the tissues in terms of inflammation.

Many attempts have been made to determine a threshold/cut-off oPMN count that can discriminate between those with and without active periodontal inflammation (Khoury et al. 2020). A mean count of 45,000 oPMNs/mL in oral samples has been reported for healthy individuals (Hans, Goswamy, and Hans 2020; Rijkschroeff et al. 2016). Therefore, delineating patients based on the threshold of 5.0×10^4 oPMNs/mL oral rinse can potentially provide a convenient method for screening patients for ongoing oral inflammation compared to traditional BoP-dependent methods in a non-dental setting. Although extremely promising, the direct quantification of oPMNs in rinse samples is limited by the need for laboratory equipment and trained technicians, which is not feasible in point-of-care settings, such as dentist or physician offices. Therefore, the NEA test, which relies on the simple colourimetric strip method described in this study, represents an attractive alternative that can provide an estimate of the overall oPMN counts in any given oral rinse sample and differentiate between healthy and gingival inflammation consistent with gingivitis. Thévenot et al. (2016). have recently demonstrated the success of a comparable approach for measuring and quantifying PMNs in ascitic fluid, which can aid the diagnosis of spontaneous bacterial peritonitis.

This NEA strip test, designed to detect a cut-off of 5.0×10^4 oPMNs/mL, exhibited high sensitivity and NPV in detecting elevated ONC. Specifically, in our first cohort, the test demonstrated a sensitivity of 91.43% and an NPV of 87.50% for identifying periodontal pockets exceeding 5 mm in depth. Additionally, for CAL > 5 mm, the sensitivity was 93.55% and the NPV was 91.67%. These findings are in line with our ONC findings and similarly indicate that the NEA strip test is reliable for ruling out periodontal conditions characterized by deeper pockets and more severe attachment loss, both of which are risk factors for further periodontal destruction and long-term tooth loss (Donos 2018). Nonetheless, the test's moderate specificity and

PPV necessitate confirmatory clinical examination following positive test results.

For measures of BoP, whose absence can be used to confirm stability and health (Lang et al. 1990), we suggest that, at the least, negative test results can be relied upon to confirm that the periodontal tissues are healthy and non-inflamed. Alternatively, when a positive test is obtained, more careful intra-oral assessment or reassessment must be done while the patient is still in the dentist's office. Indeed, rapid screening platforms at the point of care can help detect signs of oral inflammation when no clear clinical or visible signs and/or symptoms are present. We suggest that, at times, clinical or visible signs/symptoms of inflammation can be present but might be missed depending on various factors that might affect the precision of a clinical examination. However, with the POCT proposed here, should testing demonstrate elevated levels of ONC but absent clinical findings, then re-examination within a month can be done, which could lead to clinically important findings that might become apparent with time. The availability of such visible and rapid test results could aid in the acceptance of referrals to dental offices and periodontists by allowing patients to observe the test results. This approach has been used with intra-oral photographs and the acceptance of other dental therapies (Ahmad 2009).

In the multicentre aspect of the study, we provided data to confirm the potential benefits of using the NEA test as a screening tool for oral inflammation within a general dental clinic practice model. We showed that negative and low test strip results correspond to ONCs $<5.0\times10^4$ oPMN/mL oral rinse characteristics for a healthy periodontium with a mean BoP of <10%. Medium or high results indicated an ONC of $>5.0\times10^4$ oPMN/mL mouth rinse and a BoP ≥10%. The NEA test showed strong discriminatory ability, as is evident by its robust area under the ROC curve. Compared to MMP-8 in saliva, which is emerging as a potential POCT for periodontal diseases (sensitivity ~63%, specificity ~84) (Wei et al. 2024; Lähteenmäki et al. 2022), the NEA strip test demonstrated similar, if not better, performance but with the advantages of simplicity and determining the degree of inflammation and early detection. In addition, when only negative or high test results were considered, the test exhibited even higher performance metrics, indicating its reliability in distinguishing between negative and heightened degrees of inflammation. The coherence across studies reinforces the reliability of the NEA test, emphasizing its potential for routine screening in dental practices and possibly other venues, as noted above.

Notably, none of the 375 patients reported discomfort during the test, emphasizing the non-invasive nature of the test. Operator feedback revealed a high level of agreement regarding the ease of applying the sample, interpreting the results and following the instructions. Minimal errors in use have been reported, further highlighting the simplicity and effectiveness of the test in real-world dental settings.

In this study, several limitations must be acknowledged. The study was designed to investigate the test's accuracy in a cross-sectional manner. Further large-scale field trials with longitudinal study designs and extended follow-up periods monitoring the test's performance over time, particularly changes in periodontal inflammation and predicting disease progression, would provide deeper insights into the long-term utility and validity of this POCT. Furthermore, this innovative strip test has the potential to rapidly assess oral inflammation of patients across various medical conditions linked to increased risk of periodontal disease. However, the exclusion of medically compromised patients in our current study limits the applicability of the findings to generally healthy populations. This necessitates further studies to investigate the efficacy and safety of the strip test in medically compromised populations, such as those with chronic systemic diseases or immunocompromised conditions, or those on medications that may affect periodontal health. In light of this, future investigations exploring the relationship between the ONC, as reflected by our strip test, and the PISA score as a measure widely accepted in studies linking periodontitis and systemic diseases, are imperative. These studies would provide a more comprehensive understanding of the strip test's utility and reliability across diverse patient groups and enhance its potential for broader clinical application. But along these lines, should this POCT be shown to be reliable enough to reduce the need for invasive testing, which in some patients can be problematic as discussed earlier, the overall experience of patients in such groups might be improved.

5 | Conclusion

In conclusion, the NEA strip test is a significant advancement in the non-invasive detection of periodontal inflammation. It offers a promising tool for early diagnosis and monitoring, enhancing both clinical and research applications in periodontal health. Importantly, it can be used reliably to demonstrate the presence of healthy tissues, an important measure when assessing the efficacy or completeness of periodontal therapy in any given patient.

Author Contributions

All authors contributed to the study. O.E., M.G. and H.T. conceptualized the study. C.S. and E.A.B. performed the laboratory work. O.E. analysed the results and prepared the original draft of the manuscript. M.G., O.E., E.A.B., H.T., S.B.L., T.E.V.D. and S.S. reviewed and edited the subsequent drafts of the manuscript.

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Conflicts of Interest

M.G. has a patent Rinse test for oral inflammation licensed to Oral Science International Inc. M.G., S.B.L., T.E.V.D. and H.T. serve as consultants and members of the Scientific Advisory Board for Oral Science International Inc.

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.