Xue *et al.*
European Journal of Medical Research (2024) 29:527
https://doi.org/10.1186/s40001-024-02111-z

European Journal
of Medical Research

RESEARCH

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Analyses of gingival papilla blood flow via color doppler flow imaging and micro-flow imaging in patients with advanced periodontitis: a clinical pilot study

Fei Xue[1†], Bin-Zhang Wu[1†], Rui Zhang[2†], Yong Zhang[1*] and Nan Li[3*]

Abstract

Background Research investigating the potential link between gingival microvascular blood flow and inflammatory status is scarce. This study aims to assess color doppler flow imaging (CDFI) and micro-flow imaging (MFI) as tools for the assessment of gingival papilla blood flow (GPBF) and to examine their diagnostic utility as a noninvasive means of detecting gingival bleeding.

Methods CDFI and MFI were used to assess the GPBF grade (0–4) of 140 anterior gingival papilla sites in advanced periodontitis patients. Correlations between GPBF grades and periodontal characteristics were examined, and diagnostic performance as a means of predicting bleeding on probing (BOP) was examined using receiver operating characteristic curves.

Results GPBF grades 0 and 1 assessed by the MFI were 14.29% and 15.71% respectively, lower than the 28.57% and 24.29% assessed by the CDFI. In contrast, MFI detected a higher frequency of GPBF grade 2 sites (40.71%) relative to CDFI (22.14%). The CDFI and MFI provided consistent results in 62.14% of the sites, while the MFI demonstrated higher ratings in rest 37.86% of the sites. A significant positive correlation was detected between GPBF grade and the modified gingival index (MGI), bleeding index (BI), BOP, and probing depth (PD). It showed high accuracy for CDFI or MFI to diagnosing BOP with a sensitivity of 80.51% and 96.43% and a specificity of 77.27% and 57.14%, respectively. Area under the receiver operator characteristic curve values when predicting BOP based on the GPBF grade determined using CDFI and MFI approaches 0.887 (95% CI 0.833–0.942) and 0.917 (95% CI 0.862–0.972), respectively, and there were no significant differences between these values ($Z = -1.502$, $p = 0.133$).

Conclusions Both MFI and CDFI can be employed for the evaluation of GPBF, and MFI is better suited to detecting mild inflammation.

Trial registration ChiCTR2200066021 (Date of registration: 22/11/2022).

Keywords Blood flow, Color doppler flow imaging, Micro-flow imaging, Gingival papilla, Periodontitis, Ultrasonograph

†Fei Xue, Bin-Zhang Wu and Rui Zhang have contributed equally to this work.

*Correspondence:
Yong Zhang
zhangyong_1019@163.com
Nan Li
linan301@vip.126.com
Full list of author information is available at the end of the article

Background

Periodontitis is a chronic disease that is highly prevalent worldwide. If left untreated, it can affect not only the health and stability of teeth by damaging the attachment of periodontal tissues, but also the risk and severity of various systemic diseases [1]. Early diagnosis is critical to the effective treatment or prevention of periodontitis, and inflammation of the gingiva is one of the earliest symptoms of this condition [2]. This inflammation leads to the dilation of local capillaries and increased capillary permeability such that inflammatory cells can infiltrate the gingiva, promoting localized swelling [3].

The clinical methods available for detecting the severity of periodontal conditions are oral examination, periodontal probing, X-ray examination, and bacterial examination [4]. Clinically, dentists often examine the color, swelling, bleeding and recession of the gingiva in conjunction with periodontal probing results that measure the depth of the periodontal pockets around the teeth in order to determine the health of the gingiva. Most parameters used at present to describe the extent of gingival inflammation, however, are subjective (bleeding index, gingival index) or are dichotomous parameters associated with bleeding on probing (BOP) [5]. Periodontal probes are also necessary to make these measurements, subjecting patients to discomfort and stress [6].

Ultrasonographic imaging can readily identify changes in blood flow. Several recent studies have successfully applied color doppler flow imaging (CDFI) as a means of assessing relative peri-implant health or inflammation and the assess blood perfusion after connective tissue graft (CTG)-based soft tissue augmentation [7, 8]. Similarly, the high-resolution contrast-free micro-flow imaging (MFI) vascular imaging technique can sensitively evaluate low-velocity and deep microvascular flow [9]. MFI strategies utilize algorithms to exclude tissue motion and to extract specific flow signals which can then be displayed in the form of colorized or monochromatic maps. MFI strategies have been successfully used to characterize blood vessels, exhibiting a high degree of predictive utility in many malignancies [10].

No prior studies to our knowledge have utilized MFI to assess periodontal soft tissue blood flow. This study was thus designed to explore the utility of CDFI and MFI-based approaches to the assessment of gingival papilla blood flow (GPBF) and to gauge their diagnostic value when identifying gingival bleeding in a non-invasive manner.

Methods

Study design

This observational cross-sectional diagnostic accuracy study received approval from the Ethics Committee of Peking University Stomatology Hospital (PKUSSIRB-202280127) and was registered in the Chinese Clinical Trial Registry (ChiCTR2200066021, 22/11/2022). Experiments were performed in accordance with the Declaration of Helsinki of 1975 (2013 revision). This study enrolled patients undergoing consultation in the Department of Periodontology of the First Clinical Division at Peking University School and Hospital of Stomatology in November to December 2022. Eligible patients were individuals at least 18 years of age suffering from severe periodontitis (Stages II–III, Grades B/C) without any periodontal treatment [11]. All patients did not have any missing teeth in the anterior region and the teeth had no fillings, restorations or orthodontic brackets. Subjects were excluded from the study if they had any of the following: (i) a history of systemic disease; (ii) taken anticoagulant or antibiotics medications within the previous 3 months; (iii) pregnant or breastfeeding woman; (iv) undergoing periodontal or orthodontic treatment; (v) implant in the anterior region; and (vi) smokers. All patients provided written informed consent to participate.

CDFI and MFI analyses

All study participants underwent ultrasound examinations in the Department of Ultrasonography of the General Hospital of the People's Liberation Army. Analyses were performed by a sonographer with over 10 years of experience. CDFI and MFI measurements of GPBF were made with a Philips ultrasound instrument (Philips Healthcare EPIQ Elite Ultrasound System Inc., Bothell, Washington, USA) and an eL18-4 high-frequency probe (20 MHz) (Fig. 1). The skilled examiner reliability was assessed within a 2-h consultation in five different patients prior to this study, with Kappa values for CDFI and MFI were 0.86 and 0.84.

For imaging, patients were directed to remain still and breathe steadily. A coupling agent (Zihui, Henan, China) was then applied to the extraoral skin, followed by the placement of the probe on the labial portion of the anterior teeth region in a transverse section (Fig. 1). The probe was maintained as stably as possible without exerting excessive force on the underlying soft tissue. Place the probe at the nasolabial groove and use B-mode ultrasound imaging to locate the position of the upper/lower incisors. Move the probe slightly up and down to select the maximum section of the gingival papilla. Image scale and gain were then adjusted as necessary to minimize background noise and to maximize sensitivity when assessing blood flow via CDFI and MFI. Screenshot images were collected and rearranged at random, after which two experienced observers blinded to patient periodontal condition performed GPBF grading for

Fig. 1 Ultrasound instruments used for these analyses. that were utilized in this study. **a** Philips ultrasound instrument. **b** eL18-4 ultrasound probe

all images. Any discrepancies between observers were resolved through consensus.

Gingival papilla blood flow grading

GPBF patterns were classified into five grades as follows (Fig. 2):

Grade 0, blood flow absent;
Grade I, dot-like blood flow;
Grade II, single line-like blood flow;
Grade III, bifurcated dendritic blood flow;
Grade IV, blood flow filling the entire gingival papilla.

Periodontal examinations

Ten gingival papillae within the region of maxillary and mandibular anterior teeth were assessed in each patient by one calibrated periodontal specialist (F.X.) blinded to ultrasound findings at the Department of Periodontology, First Clinical Division at Peking University School and Hospital of Stomatology. This specialist was responsible for assessing six clinically relevant periodontal parameters including Plaque index (PI) [12], Modified gingival index (MGI) [13], Bleeding index (BI) [14], Probing depth (PD), BOP, and clinical attachment loss (CAL). CAL and PD were measured at both the mesial and distal sites of each gingival papilla and the average values were recorded. Periodontal probing was accurate to the nearest millimetre. Intra-examiner reliability was also assessed with kappa values for PI, MGI, and BI were 0.81, 0.83, and 0.82, respectively. The correlation coefficients of agreement of PD and CAL were 0.90 and 0.92.

Statistical analysis

SPSS 24.0 (IBM, NY, USA) was used for all statistical testing. Data distributions were examined with the Shapiro–Wilk test, and are reported as means with standard deviations (SDs) or medians (Q_1, Q_3). Differences in GPBF grades as measured via MFI and CDFI were analyzed with the paired samples Wilcoxon signed rank test, while relationships between GPBF grades and periodontal parameters were assessed through Spearman's correlation analyses. The diagnostic value of GPBF when assessing BOP was evaluated using receiver operating characteristic (ROC) curves, with $p < 0.05$ as the significance threshold. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), and area under the ROC curve (AUC) were calculated.

Results

Study participant information

This study recruited 14 participants (7 males, 7 females) with a mean age of 33.71 ± 8.05 years (range: 25–49 years). In total, 140 sites were examined in these participants. For further details regarding participant characteristics, see Table 1.

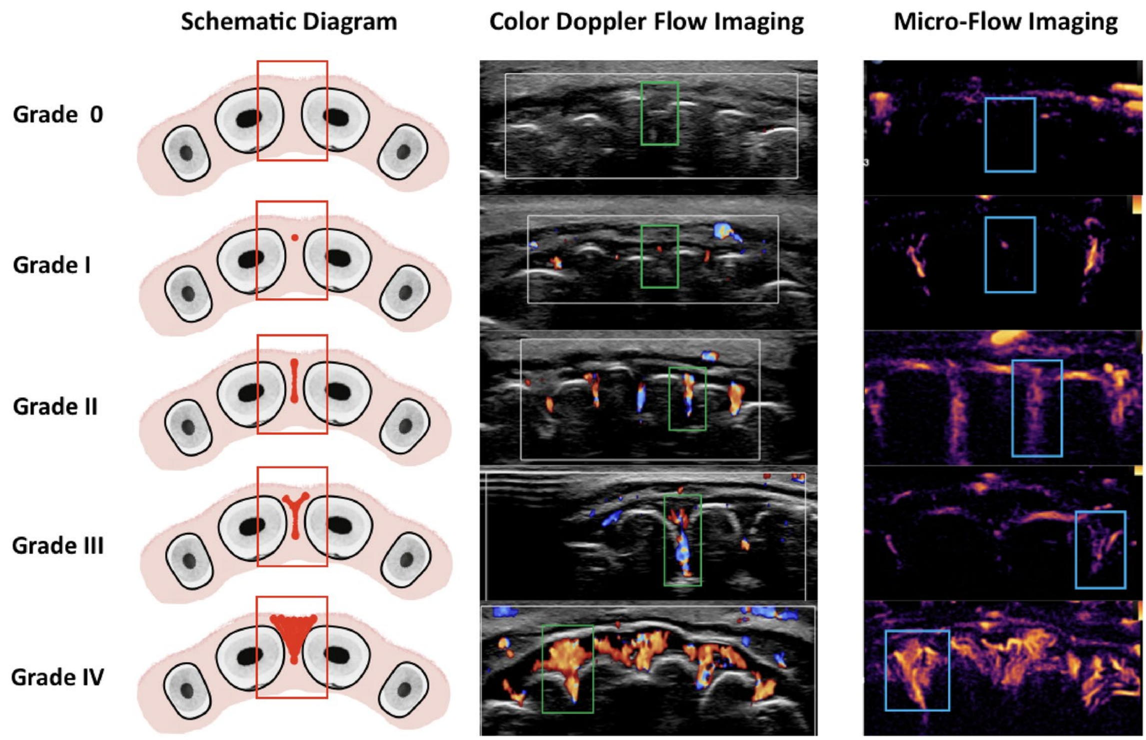

Fig. 2 Schematic overview of the use of CDFI and MFI for gingival papilla blood flow grading. A blood flow pattern is illustrated within the red box (left), while CDFI is shown in the green box (middle), and MFI is shown in the blue box (right)

Table 1 Demographic and clinical characteristics at baseline

Parameters	Values
Gender	
Male	7 (50%)
Female	7 (50%)
Age	
Median	31.5
Mean ± SD	33.71 ± 8.05
Plaque index	
Median (Q_1, Q_3)	3 (2, 3)
Modified gingival index	
Median (Q_1, Q_3)	3 (2, 4)
Bleeding index	
Median (Q_1, Q_3)	3 (2, 4)
Bleeding on probing	
+	112 (80.00%)
–	28 (20.00%)
Probing depth (mm)	
Median (Q_1, Q_3)	5 (4, 6)
Mean ± SD	5.10 ± 1.90
Clinical attachment loss (mm)	
Median (Q_1, Q_3)	5 (4, 7)
Mean ± SD	5.60 ± 2.31

SD: standard deviation

Comparisons of the assessment of GPBF using CDFI and MFI approaches

Significant differences in GPBF grading were observed when comparing the results of MFI- and CDFI-based approaches ($p < 0.0001$). Of 140 analyzed sites, CDFI respectively classified 40 (28.57%) and 34 (24.29%) as grade 0 and grade I, with these rates being significantly higher than the 20 sites (14.29%) and 22 sites (15.71%) classified as grades 0 and I by MFI. Moreover, MFI classified significantly more sites as grade II (57/140, 40.71%) as compared to CDFI (31/140, 22.14%). Overall, grading was consistent between CDFI- and MFI-based approaches for 87 sites (62.14%), whereas for the remaining 53 sites (37.86%) the grades assigned based on MFI results were higher than those based on CDFI results. A comparison of CDFI and MFI detecting gingival papilla blood flow grade is presented in Table 2 and Fig. 3.

Correlations between MFI- or CDFI-based GPBF grading and periodontal parameters

Next, correlations between these GPBF grade measurements and patient periodontal parameters were analyzed (Table 3), revealing a significant positive correlation between grades derived from CDFI or MFI approaches and parameters including MGI, BI, BOP, and

Table 2 Comparison of the capability of CDFI and MFI in detecting gingival papilla blood flow

	MFI					Total
	Grade 0	Grade I	Grade II	Grade III	Grade IV	
CDFI						
Grade 0	*20*	15	5	0	0	40
Grade I	0	*7*	25	2	0	34
Grade II	0	0	*27*	4	0	31
Grade III	0	0	0	*21*	2	23
Grade IV	0	0	0	0	*12*	12
Total	20	22	57	27	14	140

The results shown in italics above represent the same GPBF values measured by CDFI and MFI

CDFI: color doppler flow imaging; MFI: micro-flow imaging

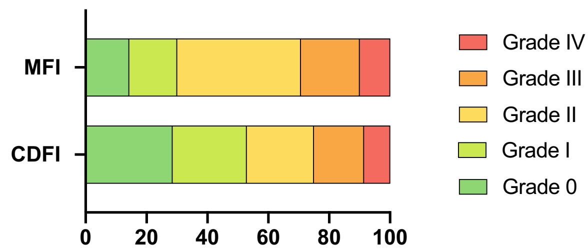

Fig. 3 Diagram highlighting the gingival papilla blood flow grade as measured by MFI or CDFI. The *X*-axis corresponds to the percentages of different grades, while the *Y*-axis denotes different methodological approaches. CDFI, color Doppler flow imaging; MFI, micro-flow imaging

Table 3 Correlation of periodontal parameters and the GPBF grade measured by CDFI or MFI

Periodontal parameters	Methods	r	*p*
Plaque index	CDFI	0.3039	0.0003
	MFI	0.3419	< 0.0001
Modified gingival index	CDFI	0.6980	< 0.0001
	MFI	0.6946	< 0.0001
Bleeding index	CDFI	0.6888	< 0.0001
	MFI	0.6918	< 0.0001
Bleeding on probing	CDFI	0.5520	< 0.0001
	MFI	0.6043	< 0.0001
Probing depth	CDFI	0.6755	< 0.0001
	MFI	0.6380	< 0.0001
Clinical attachment loss	CDFI	0.2642	0.0016
	MFI	0.3640	< 0.0001

GPBF grade: gingival papilla blood flow grade; CDFI: color doppler flow imaging; MFI: micro-flow imaging

PD. Specifically, higher GPBF grades were associated with increased MGI and BI grades, a higher PD value, and a greater chance of BOP positivity. In contrast, only weak correlations were observed between imaging-based GPBF grades and PI or CAL.

Diagnostic utility of CDFI or MFI in the detection of BOP

The Standards for Reporting Diagnostic Accuracy (STARD) flowchart for diagnosis of BOP using CDFI and MFI is displayed in Fig. 4. The diagnostic accuracy of CDFI or MFI in the detection of BOP are reported in Table 4 and the ROC curves are shown in Fig. 5. It showed high accuracy for CDFI or MFI to diagnosing BOP with a sensitivity of 80.51% and 96.43% and a specificity of 77.27% and 57.14%, respectively. The AUC values when diagnosing BOP based on CDFI and MFI grading results were 0.887 (95% CI 0.833–0.942) and 0.917 (95% CI 0.862–0.972), respectively. While the MFI-based approach offered a higher degree of diagnostic accuracy in this analysis, the difference between these two techniques was not significant ($Z = -1.502$, $p = 0.133$). The respective Youden's index values for CDFI and MFI were 0.5 and 1.5.

Discussion

Ultrasound-based approaches can enable the accurate real-time examination of periodontal tissues for diagnostic parameters [15–17]. In a prior study, Tattan et al. analyzed six parameters through both ultrasonography and direct measurement including interdental papilla height at the tooth, mid-facial soft tissue height at the tooth, soft tissue height at the edentulous ridge, mucosal thickness at the tooth and edentulous ridge, and crystal bone level at the tooth, revealing no significant difference between ultrasonography and cone–beam computed tomography measurements [7]. The ability to accurately and noninvasively detect gingival inflammation has clear clinical value. At present, BOP and BI are the two main parameters employed when measuring periodontal inflammation, yet both

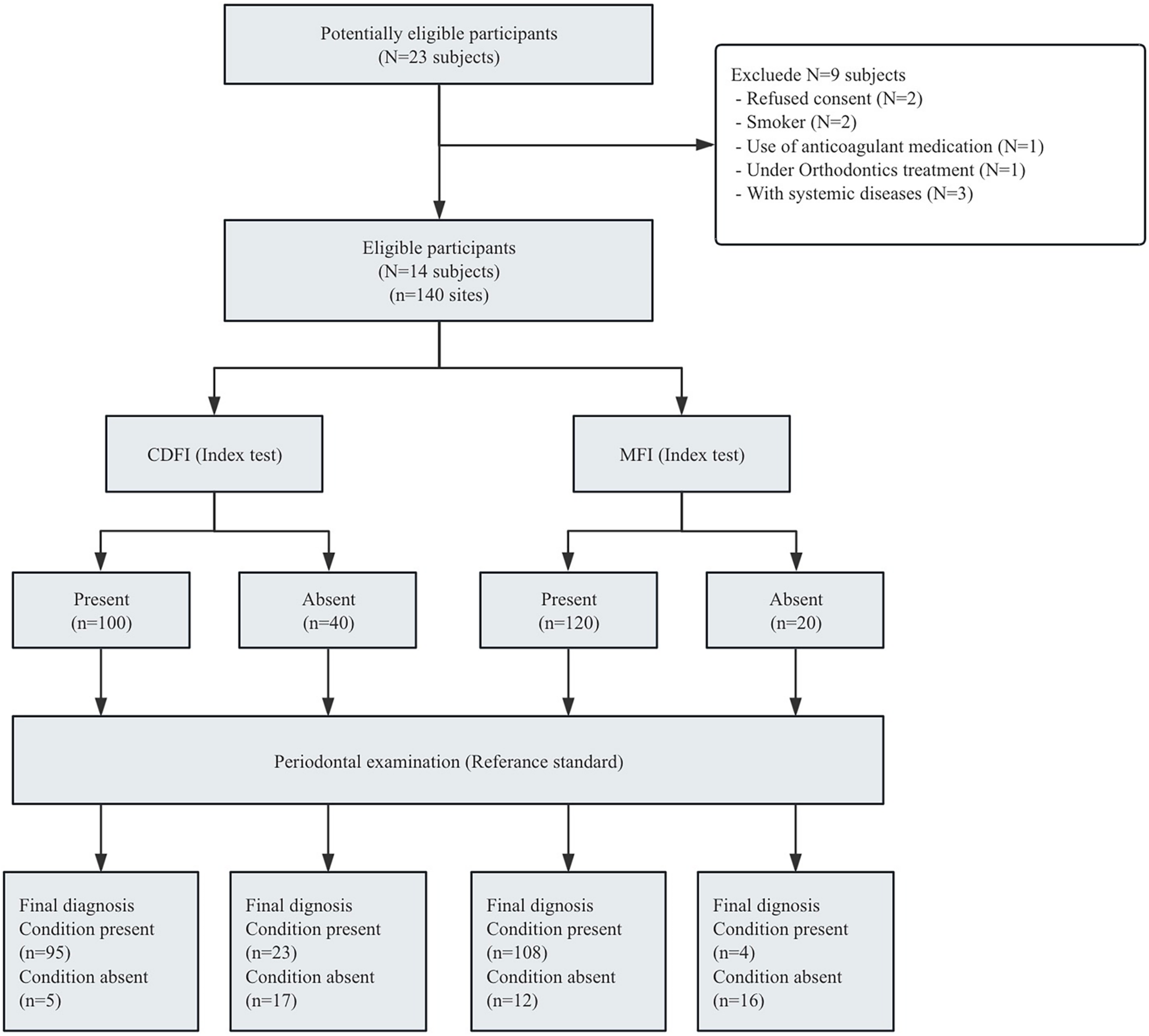

Fig. 4 Standards for reporting diagnostic accuracy flow diagram for diagnosis of BOP using CDFI and MFI

Table 4 Diagnostic accuracy of CDFI or MFI in the detection of BOP

	CDFI	MFI
Sensitivity	80.51%	96.43%
Specificity	77.27%	57.14%
PPV	95.00%	90.00%
NPV	42.50%	80.00%
PLR	3.54	2.25
NLR	0.25	0.06
AUC	0.887	0.917

CDFI: color doppler flow imaging; MFI: micro-flow imaging; BOP, bleeding on probing; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUC, area under receiver operator characteristic curve

are subjective and can be impacted by a range of factors [18]. Gingival inflammation results in the dilation and enhanced permeability of local capillaries, increasing the proximal blood flow rate and promoting the swelling and reddening of nearby soft tissue [19]. Ultrasound-based imaging strategies can detect both overall periodontal anatomy, gingival elasticity as well as associated blood flow, thereby enabling the assessment of potential tissue inflammation [20, 21]. Color flow images collected via ultrasonography can potentially be leveraged to detect even subclinical levels of inflammation. Barootchi et al. employed ultrasound scanning to assess implant-associated mid-buccal and mesial/distal regions, examining the relationship between clinical

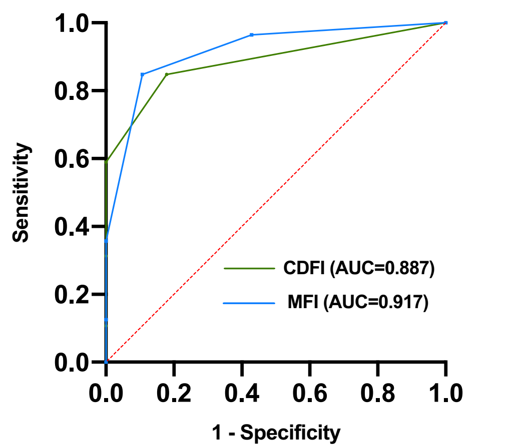

Fig. 5 ROC curves for MFI and CDFI performance when determining BOP

diagnosis and ultrasound color flow image parameters. This ultimately led them to conclude that color ultrasonography can effectively diagnose peri-implant tissue diseases and can be employed to measure the extent of clinical inflammation at implant-associated tissue sites [22]. Tavelli et al. also utilized power doppler ultrasonography to assess blood flow at implant and palatal donor sites after CTG-based soft tissue augmentation procedures, demonstrating the promise of this non-invasive approach to measuring tissue perfusion across different stages of soft tissue healing within the oral cavity [8]. While promising, these prior studies have specifically focused on ultrasound-based measurements of peri-implant blood flow, and no reports to our knowledge have similarly employed ultrasonography to measure GPBF as an index for gingival inflammation in periodontitis patients. If these findings are confirmed in future research, more detailed, timely, and accurate detection of subclinical inflammation through ultrasound can provide a non-invasive and ionizing radiation free alternative for early diagnosis, screening, and follow-up.

Doppler-based blood flow classification was initially proposed in studies of breast cancer performed in 1990, including classifications of absent, minimal, moderate, or marked flow [9]. Further studies and technological advances have expanded on this classification scheme, and breast lesions are now classified into five different patterns determined based on the shaping of the vascular network including non-vascular, linear/curvilinear, tree-like, root hair-like, and crab claw-like patterns [23, 24]. Similarly, anatomical and blood flow characteristics were herein used to define five GPBF patterns.

MFI allows for the high-resolution detection of smaller blood vessels with less suppression through the use of an enhanced algorithm that can compensate for motion blurring, adjust persistence to avoid suppressing smaller vessels, and boost vessel definition via enhancement during post-processing. This technique also employed acoustic design enhancements including an aperture designed to maximize the spatial resolution and signal strength. Han et al. examined traditional CDFI and MFI approaches, ultimately determining that MFI was able to reveal more blood vessels within liver lesions while offering a more clear overview of the distribution of blood flow, enabling the grading of blood flow in malignant liver tumors exhibiting varying degrees of differentiation [25]. Contrast-enhanced ultrasound (CEUS) is an accurate and reliable method that can significantly increase the sensitivity in assessing low-velocity and micro flow [26, 27]. Nevertheless, CEUS imaging technique requires the intravenous injections of contrast agents and cannot be done at any time. Zhang et al. further compared the diagnostic utility of MFI to that of and CEUS when evaluating carotid plaques for neovascularization, ultimately determining that both of these techniques yielded consistent findings, offering clinical utility in this diagnostic context [28].

This study is the first to our knowledge to examine GPBF via CDFI and MFI in patients with periodontitis. In this study, the sensitivity of CDFI and MFI in the diagnosis of BOP was very high, with 80.51% and 96.43%, respectively, and the specificity was 77.27% and 57.14%, respectively. This offers technical possibilities for non-invasive examination of gingival bleeding. The overall grading agreement between these two techniques was 62.14%, and MFI can detect smaller blood vessels such that it is less likely to result in underdiagnosis, thereby offering predictive value more similar to that of other evaluated indicators such as BI. The receiver operating characteristic (ROC) curve was used to evaluate the performance of CDFI and MFI in diagnosing BOP, and the AUC was 0.917 and 0.887, respectively, showing good diagnostic efficiency. Both CDFI and MFI have the potential to diagnose BOP noninvasively. In this study, we observed a weak correlation between GPBF and both PI and CAL. A potential explanation for this finding is that PI and CAL may not be the primary indicators of disease activity. For instance, plaque accumulation on the tooth surface does not necessarily induce rapid inflammatory stimulation of the gingiva. There are situations where CAL is altered (reduced periodontium) yet the patient may still achieve periodontal stability and are classified as healthy. Conversely, a substantial correlation was found between GPBF and several clinical parameters such as GI, BI, BOP, and PD. The relationship suggests that they could act as more perceptive markers of inflammatory activity within the periodontium.

There are several limitations to these analyses. First, this was a small pilot study, and correlation analyses were not conducted to examine relationships between blood flow parameters and soft tissues exhibiting differing levels of inflammation, instead focusing only on the anterior region for the purposes of quality control. The shape and sizing of extant ultrasound probes are not currently compatible with the completion of lingual–palatal measurement analyses, highlighting the need for the design of a smaller probe better suited to oral use. Moreover, the clinical implementation of the CDFI and MFI approaches will be subject to a learning curve and dependent on the technical expertise of the operator. Future work will need to expand on the sample size and applications explored herein, for example by validating relationships between ultrasonographic findings and histological results. These approaches will ultimately help to validate the clinical utility of this analytical strategy as a means of between individuals with healthy periodontal tissue, gingivitis, and different stages of periodontitis. The future application of CDFI and MFI may 1 day help to detect gingival inflammation during its early stages or protect against recurrent periodontitis [29].

Conclusions

In summary, these findings demonstrate that as the GPBF severity grade increases, there is a corresponding increase in clinical parameters associated with gingival inflammation. As such, this GPBF grade may hold value as a diagnostic reference for BOP in periodontitis patients. Of the two tested imaging strategies, MFI exhibited greater diagnostic sensitivity. However, both MFI and CDFI can be successfully used to assess the severity of inflammation associated with the gingival papilla area in a non-invasive, cost-effective and accurate manner, ultimately facilitating a more accurate diagnosis of the patient.

Abbreviations

CDFI	Color doppler flow imaging
MFI	Micro-flow imaging
GPBF	Gingival papilla blood flow
BOP	Bleeding on probing
MGI	Modified gingival index
BI	Bleeding index
PD	Probing depth
CTG	Connective tissue graft
PI	Plaque index
CAL	Clinical attachment loss
SDs	Standard deviations
ROC	Receiver operating characteristic
PPV	Positive predictive value
NPV	Negative predictive value
PLR	Positive likelihood ratio
NLR	Negative likelihood ratio
AUC	Area under ROC curve
CEUS	Contrast-enhanced ultrasound

Acknowledgements
The authors do not have any financial interest in the companies whose materials are included in this article.

Author contributions
F.X., BZ.W. and N.L. conceptualized the overall strategy. F.X., BZ.W., and Z.R. equally contributed to the clinical translation and implementation, and preparation of the manuscript and figures. F.X., BZ.W. and N.L. collected the clinical data. R.Z. and Y.Z. performed literature search and analyzed data. F.X. was a major contributor in writing the manuscript and N.L. and Y.Z. revised and edited the manuscript. All authors read and approved the final manuscript.

Funding
The study is supported by Peking University School and Hospital of Stomatology (PKUSS20200105, PKUSSNCT-20B10).

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study received approval from the Ethics Committee of Peking University Stomatology Hospital (PKUSSIRB-202280127) and was registered in the Chinese Clinical Trial Registry (ChiCTR2200066021). Experiments were performed in accordance with the Declaration of Helsinki of 1975 (2013 revision). Written informed consent for participation was obtained from each subject recruited in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]First Clinical Division, Peking University School and Hospital of Stomatology & National Center for Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices, 37A Xishiku Street, Xicheng District, Beiing 100034, People's Republic of China. [2]Third Clinical Division, Peking University School and Hospital of Stomatology & National Center for Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices, Beijing, People's Republic of China. [3]Department of Ultrasound, The First Medical Center, Chinese PLA General Hospital, No.28 Fuxing Road, Haidian District, Beijing 100853, People's Republic of China.

Received: 18 May 2024 Accepted: 15 October 2024
Published online: 02 November 2024

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