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Monitoring Role of Non-invasive Examinations on the Clinical Efficacy of Photodynamic Therapy for Oral Potentially Malignant Disorders

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Objective: *To assess the clinical efficacy of 5-aminolaevulinic acid photodynamic therapy (5-ALA-PDT) in treating oral potentially malignant disorders (OPMDs) and investigate the utility of toluidine blue staining and autofluorescence examination for monitoring the efficacy of 5-ALA-PDT.*

Methods: *A prospective cohort study was conducted, including 75 OPMDs patients who underwent 5-ALA-PDT and follow-up observation. The patients' lesion size and clinical presentation were recorded to evaluate the clinical efficacy of 5-ALA-PDT. Toluidine blue staining and autofluorescence examination were performed as auxiliary monitoring methods, aiming to assess their diagnostic capabilities as non-invasive examinations for detecting pathological oral epithelial dysplasia (OED) and explore their monitoring value for the clinical efficacy of 5-ALA-PDT.*

Results: *Toluidine blue staining showed a sensitivity of 62.2% and a specificity of 42.9% for diagnosing OED, whereas autofluorescence examination showed a sensitivity of 67.2% and a specificity of 64.3%. The parallel combination of both examinations increased the sensitivity to 77.0%, whereas the series combination increased the specificity to 71.4%. After 5-ALA-PDT, 38.7% of patients with OPMDs achieved complete remission, with an overall response rate of 92%. Persistent positive toluidine blue staining after 5-ALA-PDT treatment was significantly associated with treatment failure. The clinical efficacy of 5-ALA-PDT gradually decreased in patients with aggravation, stable or improved lesions from non-invasive examinations both before and after treatment.*

Conclusion: *5-ALA-PDT demonstrates significant efficacy in treating OPMDs by effectively eliminating lesions. Toluidine blue staining and autofluorescence examination have certain diagnostic capabilities for OED and can be used for monitoring efficacy during 5-ALA-PDT treatment.*

Keywords: *autofluorescence imaging, oral potentially malignant disorders, photodynamic therapy, toluidine blue*

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Oral potentially malignant disorders (OPMDs) are a group of diseases occurring in the oral mucosa with the potential for malignant transformation, including oral leucoplakia (OLK), oral erythroplakia (OEK), oral submucous fibrosis, oral lichen planus (OLP) and oral

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lichenoid lesions.[1] The overall prevalence of OPMDs is approximately 4.47%,[2] with an overall malignant transformation rate of 7.9%.[3] It is estimated that 70% to 90% of oral squamous cell carcinomas arise from OPMDs.[4] Therefore, the diagnosis, treatment, monitoring of malignant transformation and follow-up management of OPMDs are crucial for preventing the occurrence and development of oral cancer and improving patient survival rates.

Photodynamic therapy (PDT) is a treatment method that involves the application of photosensitisers locally or systemically, which accumulate in abnormal proliferating tumour tissue. Subsequent irradiation of the tissue with a specific wavelength light source induces photobiological reactions, generating a large amount of reactive oxygen species and destroying tumour cells.[5-7] PDT has gradually become an important therapeutic approach for the treatment of tumours and precancerous lesions due to its high selectivity, minimal damage and rapid recovery time.[8,9] 5-aminolevulinic acid (5-ALA) is the most commonly used photosensitiser in treating OPMDs.[10,11] Currently, there are more than 10 studies worldwide exploring the efficacy of 5-ALA-PDT in treating OPMDs, but none have investigated the monitoring ability of non-invasive examinations for the efficacy of 5-ALA-PDT.

Methods for the diagnosis and monitoring of malignant transformation in OPMDs include clinical, histopathological and auxiliary examinations. The accuracy of clinical examination relies on the experience of the dental practitioner and can lead to missed or misdiagnosed cases. Histopathological examination is limited by factors such as trauma, time consumption and patient discomfort, making it difficult to use as a long-term monitoring method in clinical follow-up management. In recent years, many researchers have been dedicated to finding non-invasive or minimally invasive auxiliary examination methods to alleviate patient suffering and assist in clinical diagnosis and prognosis assessment.[12,13]

Toluidine blue, as an acidic metachromatic dye, has a strong affinity for nucleic acids, making it easier to stain tissues with epithelial dysplasia or tumour tissue.[14,15] VELscope (LED Dental, Vancouver, Canada) is the most widely used autofluorescence examination device, which emits blue light to excite endogenous fluorophores and is used to differentiate normal, abnormal proliferative and tumour tissue.[16,17] Studies have shown that these two non-invasive examinations have significant auxiliary value in the early diagnosis of OPMDs.[18-20] However, there is currently no research exploring their application in monitoring during 5-ALA-PDT.

This study prospectively collected data and conducted follow-up management of patients with OPMDs receiving 5-ALA-PDT to explore its clinical efficacy in treating OPMDs. Additionally, the study incorporated toluidine blue staining and autofluorescence examination into the assessment of 5-ALA-PDT efficacy and follow-up observation, aiming to provide more objective and convenient methods for monitoring during treatment.

Materials and methods

Patient inclusion and exclusion

This study was a prospective follow-up cohort study conducted from January 2016 to August 2023 at the Department of Oral Diseases, Peking University School of Stomatology. Patients diagnosed with OPMDs based on clinical and histopathological assessments, who underwent 5-ALA-PDT treatment and received non-invasive examinations (toluidine blue staining, autofluorescence examination) during follow-up, were included. The clinical diagnostic criteria for OPMDs followed the WHO consensus,[1,21] with histopathological results serving as the diagnostic gold standard. Patients with severe systemic infections, uncontrolled systemic diseases, severe mental illness or intolerance to or the inability to complete PDT treatment were excluded.

Baseline data collection

At the initial visit, patients' clinical basic information was collected, including sex, date of birth, medical history, smoking history, alcohol consumption history, betel nut chewing history, symptoms and course of disease. Patients underwent a comprehensive examination of the oral mucosa and routine oral examination, and the nature, location, number, size (cm^2), clinical type, oral hygiene status, local irritants and initial clinical diagnosis of the lesions were recorded. Lesions were photographed using a digital single-lens reflex camera. Clinical types of OLK included homogeneous and non-homogeneous (verrucous type, granular type, ulcerative type). Other OPMDs were not classified into subtypes.

Toluidine blue staining examination

Patients were instructed to rinse their mouths with water for 30 seconds. Using a dry cotton swab, the liquid on the surface of the lesion was wiped away. A new cotton swab was dipped in 1% acetic acid solution and applied

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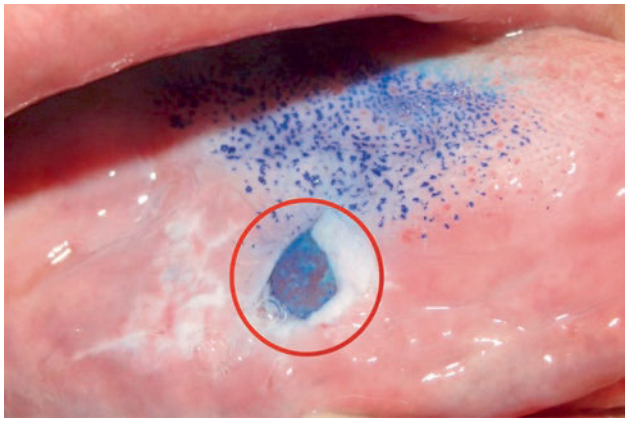

Fig 1 Positive toluidine blue staining on the right ventral tongue, ulcerative type oral leucoplakia with moderate epithelial dysplasia.

to the lesion surface for 30 seconds to remove the saliva film. Subsequently, another cotton swab was dipped in 1% toluidine blue solution and applied to the lesion surface for 30 seconds. During this period, patients were instructed not to close their mouths, and then to rinse their mouths with water three times. A cotton swab dipped in 1% acetic acid solution was used to wipe the lesion surface to remove mechanical staining, and finally, patients were asked to rinse their mouths with water for 30 seconds. Lesions that still showed patchy staining after bleaching were judged and recorded as positive. Lesions without staining were recorded as negative, whereas those with light staining that was difficult to determine were considered suspicious (Fig 1).

Autofluorescence examination

The examination was conducted in a dark environment. The examiner held a VELscope autofluorescence instrument, allowing fluorescence to be projected vertically onto the lesion site. The lesion was observed through the eyepiece, and the fluorescence photograph results were stored using an iPod touch 4. Examination results were recorded as negative, suspicious or positive. The judgment criteria for autofluorescence detection results were that normal tissue exhibited a faint green fluorescence after fluorescence irradiation, indicating a negative result. Positive results were characterised by the absence of abnormal black fluorescence in irregular, asymmetrical shapes after fluorescence irradiation, with normal green fluorescence observed in the contralateral lesion-free area. Suspicious results were recorded for cases with fluorescence attenuation that was difficult to determine as physiological or abnormal (Fig 2).

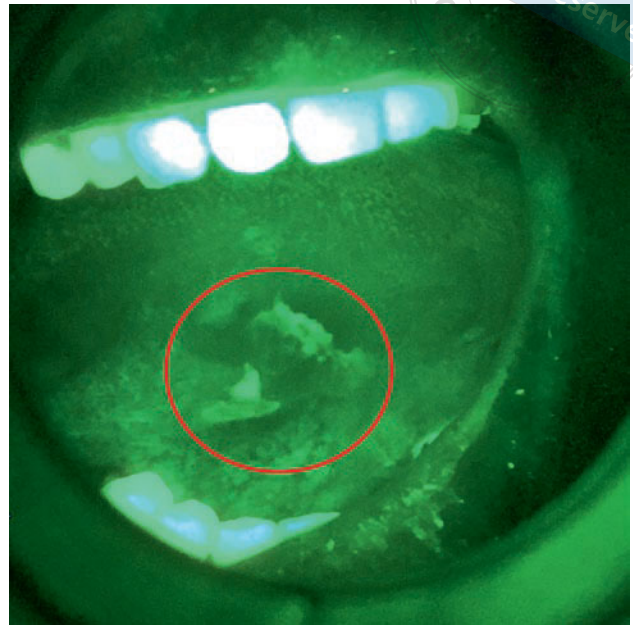

Fig 2 VELscope image of the left ventral tongue (granular type oral leucoplakia with moderate oral epithelial dysplasia) showed fluorescence visualisation loss.

Histopathological examination

For lesions that did not regress after local irritation was removed for 2 weeks, the most typical area of the lesion was selected for a local biopsy or excision biopsy specimen to be taken. Tissue samples were fixed in formalin solution and embedded in paraffin after immersion. After slicing, haematoxylin-eosin staining was performed, followed by microscopic examination. Oral pathologists made histopathological diagnoses based on disease diagnostic criteria. For cases of oral epithelial dysplasia (OED), the degree was classified into three categories according to WHO standards: mild, moderate and severe. If there were two or more degrees of OED, the result was based on the more severe degree. Lesions showing suspicious early carcinoma or early infiltration were classified as oral cancer.

Clinical typing, toluidine blue staining results, autofluorescence examination results and histopathological examination results were primarily determined by one physician, with two additional physicians providing secondary review and confirmation.

Follow-up visits and data collection

During each visit, changes in the patient's general condition were first recorded, including symptoms, habits and systemic diseases. Before each 5-ALA-PDT treat-

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ment, clinical examination, toluidine blue staining examination and autofluorescence examination were conducted, and the results were recorded and retained with photographs. Changes in non-invasive examination results compared to baseline status were recorded as reduction (positive to suspicious/positive to negative/suspicious to negative), no change or aggravation (negative to suspicious/negative to positive/suspicious to positive).

According to the expert consensus on 5-ALA-PDT for oral potentially malignant disorders by the Chinese Stomatological Association,[22] standardised 5-ALA-PDT treatment was performed. A 20% concentration of 5-ALA gel was topically incubated for at least 2 hours. After thorough fluorescence distribution was confirmed by Wood's lamp, local anaesthesia was administered. A 635-nm, 600-mw laser light source was used with the fibre optic head positioned 2 cm from the lesion surface and perpendicular to the lesion area. The diameter of the irradiation spot was adjusted to 1 cm, and each spot was irradiated for 3 to 4 minutes to ensure a light flux within the range of 50 to 150 J/cm^2.

Treatment intervals were adjusted based on the severity of the patient's condition and treatment response, and treatment was administered every 1 to 3 weeks until the lesion disappeared or until an oral mucosal disease specialist deemed it appropriate to stop. The number of treatments, treatment parameters and treatment response were recorded.

A follow-up visit was scheduled 2 to 4 weeks after the end of the PDT course. Patient symptoms, clinical manifestations, lesion size, toluidine blue staining examination and autofluorescence examination results were recorded, and photographs were taken and retained. The clinical efficacy of PDT was assessed based on the size of the lesion and clinical presentation.

Evaluation criteria

Diagnostic ability of non-invasive examinations for detecting OED

Using the presence of OED in histopathological diagnosis as the gold standard, the sensitivity, specificity, accuracy, positive predictive value, negative predictive value and Youden index of toluidine blue staining and autofluorescence examination were calculated. Receiver operating characteristic curves were plotted, and the area under the curve (AUC) was calculated.

In a parallel test, if either of the two non-invasive examination results is positive, it is considered positive; both results must be negative to be considered negative.

In a series test, if one of the two non-invasive examination results is negative, it is considered negative; both results must be positive to be considered positive.

$$Sensitivity = \left(\frac{True\ Positives}{True\ Positives\ +\ False\ Negatives}\right) \times 100\%$$

Representing the ability of a diagnostic method to correctly detect actual patients.

$$Specificity = \left(\frac{True\ Negatives}{True\ Negatives\ +\ False\ Positives}\right) \times 100\%$$

Representing the ability of a diagnostic method to correctly detect actual non-patients.

$$Positive\ Predictive\ Value = \left(\frac{True\ Positives}{True\ Positives\ +\ False\ Positives}\right) \times 100\%$$

Indicating the proportion of truly diseased patients among those indicated as diseased by a diagnostic method.

$$Negative\ Predictive\ Value = \left(\frac{True\ Negatives}{True\ Negatives\ +\ Negatives}\right) \times 100\%$$

Indicating the proportion of truly non-diseased individuals among those indicated as non-diseased by a diagnostic method.

$$Accuracy = \left(\frac{True\ Positives\ +\ True\ Negatives}{Total\ cases}\right) \times 100\%$$

Indicating the proportion of correctly diagnosed patients and non-patients among all individuals undergoing diagnosis.

Clinical efficacy evaluation of 5-ALA-PDT

Clinical efficacy evaluation was conducted 2-4 weeks after the completion of 5-ALA-PDT treatment, according to the following criteria:

- Complete response (CR): The target lesion was found to have disappeared upon clinical examination.
- Partial response (PR): Compared to baseline, the size of the lesion has decreased by ≥ 20% or there is a transition from a severe clinical type to a milder clinical type, or toluidine blue staining or autofluorescence examination result has turned negative.
- No response (NR): The lesion area has decreased by < 20% or has increased in size, or the clinical type has aggravated, or toluidine blue staining or autofluorescence examination result has turned positive.

The overall response (OR) rate was ((CR + PR) / (CR + PR + NR)) × 100%.

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Table 1 Demographics of patients who received 5-ALA-PDT.

Variable		Male	Female	Total
Total		35 (46.7%)	40 (53.3%)	75 (100.0%)
Age (year)	Mean ± SD	51.99 ± 13.96	56.25 ± 12.25	54.26 ± 13.16
Systemic diseases	Yes	21 (60.0%)	25 (62.5%)	46 (61.3%)
	No	14 (40.0%)	15 (37.5%)	29 (38.7%)
Smoking	Yes	25 (71.4%)	0 (0.0%)	25 (33.3%)
	No	10 (28.6%)	40 (100.0%)	50 (66.7%)
Drinking	Yes	21 (60.0%)	3 (7.5%)	24 (32.0%)
	No	14 (40.0%)	37 (92.5%)	51 (68.0%)
Disease classification	OLK	33 (94.3%)	31 (77.5%)	64 (85.3%)
	OEK	2 (5.7%)	6 (15.0%)	8 (10.7%)
	Other OPMDs	0 (0.0%)	3 (7.5%)	3 (4.0%)
Clinical types of OLK	Homogenous	17 (51.5%)	7 (22.6%)	24 (37.5%)
	Non-homogenous	16 (48.5%)	24 (77.4%)	40 (62.5%)
Lesion location	High-risk areas[a]	20 (57.1%)	21 (52.5%)	41 (54.7%)
	Others	15 (42.9%)	19 (47.5%)	34 (45.3%)
OED	No	7 (20.0%)	7 (17.5%)	14 (18.7%)
	Mild	10 (28.6%)	10 (25.0%)	20 (26.7%)
	Moderate	10 (28.6%)	15 (37.5%)	25 (33.3%)
	Severe	8 (22.9%)	8 (20.0%)	16 (21.3%)
Clinical efficacy of 5-ALA-PDT	CR	12 (34.3%)	17 (42.5%)	29 (38.7%)
	PR	22 (62.9%)	18 (45.0%)	40 (53.3%)
	NR	1 (2.9%)	5 (12.5%)	6 (8.0%)

[a]High-risk areas refer to the margin and ventral surface of the tongue and floor of the mouth

SD, standard deviation.

Statistical analysis

The statistical analysis was performed using SPSS version 26.0 software (IBM, Chicago, IL, USA). Normally distributed continuous variables were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables were presented as median (Md) and interquartile range (P25, P75). Categorical variables were presented as frequency and percentage.

For continuous variables, normality and homogeneity of variance were assessed. If the assumptions were met, a Student *t* test or analysis of variance (ANOVA) was used for between-group comparisons. If the assumptions were violated, a Mann-Whitney U test or Kruskal-Wallis test was applied. The between-group comparison of categorical variables and correlation analysis were conducted using a chi-square test. All reported *P* values were based on two-tailed tests, with the level of statistical significance set at $P < 0.05$.

Results

Demographic information

A total of 75 patients were included, with 35 men and 40 women, yielding a male-to-female ratio of approximately 1:1.14. The mean age was 54.26 ± 13.16 years. Among them, 64 cases were diagnosed as OLK and eight as OEK, and the remaining three cases included oral lichen planus, oral lichenoid lesions and chronic discoid lupus erythematosus, respectively. Among OLK patients, 24 cases were of the homogeneous type and 40 cases were non-homogeneous. Baseline histopathological examination revealed 14 cases with no OED, with 20 cases of mild, 25 cases of moderate and 16 cases of severe OED. Patients' basic information is summarised in Table 1.

The 75 OPMD patients received between one and 23 sessions of 5-ALA-PDT, with a median of 4 (2, 6) sessions. The overall response rate of 5-ALA-PDT in

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Table 2 Relationship between baseline results of two non-invasive examinations.

Non-invasive examination results		Autofluorescence examination		Total
		Positive	Negative	
Toluidine blue staining	Positive	37	9	46
	Negative	9	20	29
Total		46	29	75

Table 3 Correlation between baseline non-invasive examination results and clinical information.

Clinical information		Toluidine blue staining	*P* value	Autofluorescence examination	*P* value
		Positive/negative		Positive/negative	
Total		46/29	NA	46/29	NA
Disease classification	OLK	36/28	0.117	38/26	0.202
	OEK	7/1		7/1	
	Other OPMDs	3/0		1/2	
Lesion location	High-risk areas[a]	23/18	0.307	24/17	0.585
	Others	23/11		22/12	
Clinical types of OLK	Homogenous	8/16	0.004*	10/14	0.025*
	Non-homogenous	28/12		28/12	
OED	No	8/6	0.721	5/9	0.029*
	Yes	38/23		41/20	
5-ALA-PDT clinical efficacy	OR	41/28	0.396	42/27	0.780
	NR	5/1		4/2	

[a]High-risk areas refer to the margin and ventral surface of the tongue and floor of the mouth

*$P < 0.05$, statistically significant.

NA, not applicable; OR, overall response.

treating OPMDs was 92%, with 29 cases achieving CR and 40 achieving PR. Among OLK patients, the CR rate was 40.6% (26/64) and the overall response rate was 93.7% (60/64). The CR rates for ulcerative OLK (60.0%, 9/15), granular OLK (50.0%, 10/20), homogeneous OLK (25.0%, 6/24) and verrucous OLK (20.0%, 1/5) decreased in this order, though the differences were not statistically significant. In OEK patients, the CR rate with 5-ALA-PDT was 37.5% (3/8) and the overall response rate was 87.5% (7/8). There was no significant difference in the efficacy rates of 5-ALA-PDT among different disease types.

Correlation between baseline non-invasive examination results and clinical information

At baseline, 61.3% of patients tested positive for toluidine blue staining, and 61.3% tested positive for autofluorescence examination, with 49.3% showing positive results for both non-invasive examinations. The concordance rate of the two non-invasive examination results at baseline was 76%, as shown in Table 2. Comparing the baseline non-invasive examination results with clinical information revealed significant associations. Positive toluidine blue staining was notably associated with the non-homogeneous type of OLK ($P = 0.004$). Positive autofluorescence examination was significantly associated with non-homogeneous type OLK ($P = 0.025$) and the presence of OED ($P = 0.029$); however, there was no significant correlation between baseline non-invasive examination results and disease type, lesion site or efficacy of 5-ALA-PDT. The proportional relationship between the two non-invasive examination results and OLK clinical subtypes and the degree of OED is presented in Table 3 and Fig 3.

Non-invasive examination for diagnosing OED

The diagnostic performance of two non-invasive examination methods was evaluated based on the presence of OED as a positive result in histopathology (Table 4). When each non-invasive method was applied individually, autofluorescence examination demonstrated higher sensitivity (67.2%), specificity (64.3%), positive predictive value (89.1%) and accuracy (66.7%) compared to toluidine blue staining (sensitivity 62.2%, specificity 42.9%, positive predictive value 82.6%, accuracy 58.7%). However, toluidine blue staining exhibited a higher

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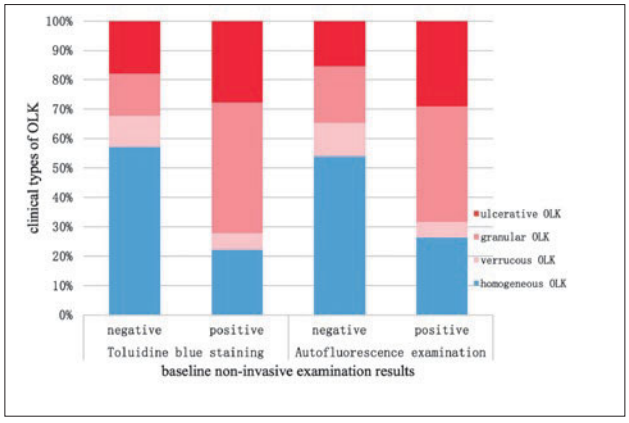

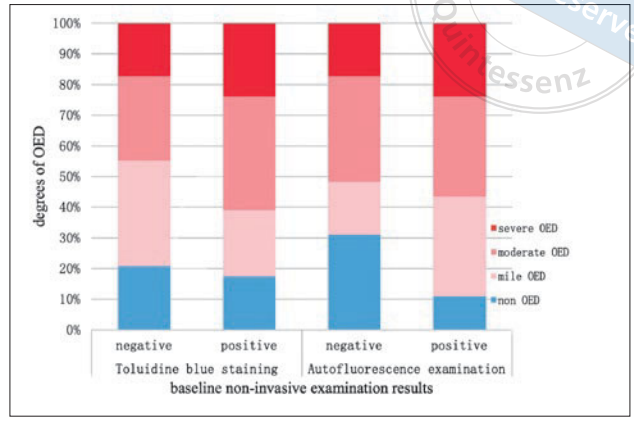

Fig 3 Relationship between baseline non-invasive examination results and clinical types of OLK and OED. At baseline, there was a significant correlation between toluidine blue staining positivity and clinical type of OLK being heterogeneous (P = 0.004), while positivity in autofluorescence examination was significantly associated with heterogeneous OLK (P = 0.029) and presence of OED (P = 0.025).

Table 4 Diagnostic performance of the two non-invasive examination methods for detecting OED.

Examination method	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy	AUC
Toluidine blue staining	62.2%	42.9%	82.6%	79.3%	58.7%	0.526
Autofluorescence examination	67.2%	64.3%	89.1%	69.0%	66.7%	0.657
Serial test	54.1%	71.4%	89.2%	73.7%	57.3%	0.628
Parallel test	77.0%	21.4%	81.0%	82.4%	66.7%	0.492

AUC, area under the receiver operating characteristic curve.

negative predictive value (79.3%) than autofluorescence examination (69.0%). When the two methods were used in combination, there was an improvement in specificity (71.4%) and positive predictive value (89.2%) for the serial test, and an increase in sensitivity (77.0%) and negative predictive value (82.4%) for the parallel test.

Correlation between changes in non-invasive examination results during follow-up and clinical efficacy of 5-ALA-PDT

All 75 patients underwent toluidine blue staining and autofluorescence examination during treatment follow-up, and the results are presented in Table 5. It was found that the NR rate in patients with positive toluidine blue staining after treatment completion was 35.7%, significantly higher than the rate in patients with negative staining (1.6%; P < 0.001). Additionally, patients with a reduction in toluidine blue staining results had the highest rate of CR, while those with no change had the highest rate of PR, and those with aggravation had the highest rate of NR, with statistically significant differences (P = 0.040). There was no significant difference in the distribution of 5-ALA-PDT efficacy between patients with positive and negative autofluorescence examination results after treatment completion (P = 0.067). However, the CR rates gradually decreased and NR rates gradually increased with decreasing reduction, no change and aggravation in autofluorescence examination results before and after treatment (P = 0.009).

During the treatment and follow-up process, 35 patients underwent toluidine blue staining and autofluorescence examination at each visit, along with an evaluation of clinical classification. The duration of clinical classification positivity (considering OLK converting from a non-homogeneous to homogeneous type or the disappearance of red lesions in OEK as negative), toluidine blue positivity and VELscope positivity were recorded and calculated statistically. The median duration of clinical classification positivity was 4.0 (2.0, 9.5) weeks, toluidine blue positivity was 4.0 (4.0, 8.0) weeks, and VELscope positivity was 4.0 (2.0, 8.0) weeks. A Mann-Whitney U test indicated no significant differences in the duration of clinical classification positivity, toluidine blue positivity and VELscope positivity (P > 0.05).

A Spearman rank correlation test revealed a significant positive correlation among the durations of clin-

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Table 5 Correlation between changes in non-invasive examination results and clinical efficacy.

Non-invasive examination results and changes		CR	PR	NR	*P* value
Toluidine blue staining after treatment	Negative	28 (45.9%)	32 (52.5%)	1 (1.6%)	< 0.001*
	Positive	1 (7.1%)	8 (57.1%)	5 (35.7%)	
Change in toluidine blue staining	Reduced	23 (50.0%)	21 (45.7%)	2 (4.3%)	0.040*
	No change	5 (20.0%)	17 (68.0%)	3 (12.0%)	
	Aggravated	1 (25.0%)	2 (50.0%)	1 (25.0%)	
Autofluorescence after treatment	Negative	23 (41.8%)	30 (54.5%)	2 (3.6%)	0.067
	Positive	6 (30.0%)	10 (50.0%)	4 (20.0%)	
Change in autofluorescence examination	Reduced	21 (56.8%)	16 (43.2%)	0 (0.0%)	0.009*
	No change	7 (21.9%)	20 (62.5%)	5 (15.6%)	
	Aggravated	1 (16.7%)	4 (66.7%)	1 (16.7%)	

*$P < 0.05$, statistically significant.

ical classification positivity, toluidine blue positivity and VELscope positivity. The correlation coefficient between the duration of clinical classification positivity and toluidine blue positivity was 0.574 ($P = 0.010$), between clinical classification positivity and VELscope positivity was 0.640 ($P = 0.008$), and between toluidine blue positivity and VELscope positivity was 0.468 ($P = 0.032$).

Discussion

This study included a total of 75 patients with OPMDs who underwent 5-ALA-PDT treatment and follow-up. Among them, 64 cases were OLK, eight were OEK and the remaining three were distributed as oral lichen planus, oral lichenoid lesions and chronic discoid lupus erythematosus. The overall response rate for 5-ALA-PDT treatment was 92%, with 38.7% achieving CR and 53.3% achieving PR. A meta-analysis conducted in 2022 showed an overall response rate of 93.7% for PDT treatment of OPMDs, with 35.3% CR among 235 cases of OLK and 91.8% among 61 cases of OEK, which is consistent with the results of this study.[23] This suggests that 5-ALA-PDT is effective in removing OPMD lesions.

The baseline results of toluidine blue staining were significantly correlated with clinical types of OLK, whereas positive results of autofluorescence examination were significantly correlated with non-homogeneous OLK and the presence of OED. This indicates that these two non-invasive examinations can be used to assess the malignancy of OPMD lesions, highlighting the importance of increased attention to patients with positive results from non-invasive examinations.

The diagnostic performance results suggested that autofluorescence examination had a slightly better diagnostic ability for detecting OED compared to toluidine blue staining. Parallel use of both methods increased the sensitivity to 77.0%, whereas series use increased the specificity to 71.4%, suggesting that combining these methods may enhance diagnostic accuracy in clinical practice. The meta-analysis results reported by Walsh et al[24] showed that the sensitivity of vital staining methods for diagnosing OPMDs or OSCC was 0.86, with a specificity of 0.68, while optical methods had a sensitivity of 0.87 and a specificity of 0.50, and the combined use of staining and optical examination methods can improve diagnostic specificity.[24] The application of non-invasive examination methods to assist in the diagnosis and detection of OPMDs is a future trend. Toluidine blue staining and autofluorescence examination have the advantages of being completely non-invasive, easy to operate and efficient, and they have certain discriminatory abilities for OED. Future research should further explore their combined application and diagnostic accuracy.

In this study, non-invasive examination methods were used to assist in the examination during 5-ALA-PDT treatment. It was found that patients with positive results for toluidine blue staining at the end of treatment had poorer efficacy of 5-ALA-PDT. The efficacy of 5-ALA-PDT decreased progressively with the reduction, stability or aggravation of non-invasive examination results both before and after treatment, indicating that non-invasive examination methods can be used to monitor the efficacy of 5-ALA-PDT treatment and assist in judging the treatment effect during follow-up.

Furthermore, we believe that non-invasive examination results can be used as one of the criteria for determining whether to discontinue 5-ALA-PDT treatment.[25] If patients' clinical lesions disappear but the non-invasive examination results remain positive, 5-ALA-PDT treatment should continue until the non-invasive examination results become negative. Conversely, if patients' clinical lesions are relatively refractory and difficult to resolve, non-invasive examination results should also be considered. If they all turn negative, this

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indicates a reduced risk of lesion malignancy, and laser ablation treatment or follow-up observation alone may be considered.

A total of 35 patients underwent non-invasive examination monitoring during each visit of the treatment follow-up. We found no significant difference in the duration of positivity between toluidine blue staining and VELscope concerning non-homogeneous clinical classifications or erythematous red lesions. This indicates that toluidine blue staining and VELscope examinations can promptly and effectively reflect changes in lesions. The results of toluidine blue staining and VELscope are distinct and easy to differentiate. Compared to distinguishing clinical manifestations of lesions, the positive or negative results of non-invasive examinations are more indicative, facilitating other non-specialised clinicians in assessing OPMDs when the clinical risk is difficult to determine.

Conclusion

This study demonstrates that 5-ALA-PDT treatment is significantly effective for OPMDs. Toluidine blue staining and autofluorescence examination have certain diagnostic capabilities for OED and can be used for monitoring during 5-ALA-PDT treatment. It is still necessary to increase the sample size and extend the follow-up period in the future in order to explore the long-term efficacy of 5-ALA-PDT treatment as well as its correlation with non-invasive examination results.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Xing Yun LIU contributed to the data analyses and manuscript draft; Drs Ying HAN and Hong Wei LIU devised the study design and revised the manuscript; Drs Qian Yun GUO, Qian WANG and Si XU contributed to the data analysis and manuscript preparation; Drs Zhe CHENG, Lei ZHANG, Yu Tian WANG, Xiang GUO, Xiao Dan LIU and Wen Wen LI contributed to the data collection and analysis; Drs Xing WANG, Shu Fang LI, Zi Jian LIU, Hong Mei CUI, Ming Xing LU and Jian Qiu JIN contributed to the data collection and patients treatment.

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