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HEAD AND NECK

Assessment of the cancerization risk for oral potentially malignant disorders by clinical risk model combined with autofluorescence and brush biopsy with DNA-image cytometry

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Abstract

Purpose To explore the feasibility of assessing the cancerization risk of oral potentially malignant disorders (OPMD) through a clinical risk model combined with autofluorescence and brush biopsy with DNA-image cytometry.

Methods We collected the baseline clinical data of 269 patients; then, performed autofluorescence, brush biopsy with DNA-image cytometry and histopathological examination. Then, we obtained the significant factors by univariate logistic analysis, constructed the clinical risk model by multiple logistic regression and selected the optimal cutoff value according to the maximum Youden index. Finally, we calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the clinical risk score ≥ cutoff value, autofluorescence and brush biopsy with DNA-image cytometry, and plotted the receiver-operating characteristic (ROC) curves and decision curve analysis (DCA).

Results The clinical risk model is represented by the formula: 1 × gender + 1.6 × age group + 1 × lesion site + 1.4 × local stimulus + 1.5 × drink. The area under the curve (AUC) was 0.83, and the optimal cutoff score was 3. The AUC indicated that the clinical risk score ≥ 3 (0.74) and autofluorescence (0.77) had a certain diagnostic values, while brush biopsy with DNA-image cytometry (0.92) displayed a good value. Besides, the DCA showed that all three tests had clinical significance.

Conclusions The cancerization risk of patients can be assessed by the clinical risk model combined with sequence application of autofluorescence and brush biopsy with DNA-image cytometry, to decide whether histopathological examination or other intervention measures should be selected.

Keywords Oral potentially malignant disorders · Autofluorescence · Brush biopsy with DNA-image cytometry · Cancerization risk · Model

Introduction

Oral cancer afflicts approximately 300,000 people worldwide every year [1]. Oral squamous cell carcinoma (OSCC) accounts for almost 90% of oral cancer [2]. Great progresses have been attained in the diagnosis and management; nonetheless, 50% OSCC patients still die within 5 years [3]. Specifically, oral potentially malignant disorders (OPMD) are associated with a statistically increased risk of progressing into OSCC [4]. The prevalence of OPMD is about 4.47% [5], with the cancerization rate of close to 4.32% [6]. OPMD patients have suffered from both physical and psychological stress due to the protracted course and long carcinogenesis. It is tough to predict the risk of cancerization for each individual, as the risk varies a range of factors, such as sexual, age and lesion-related factors [7, 8].

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Concretely, the risk scores are model-based scores that can predict the outcomes, which can assist in decision making, diagnosis and prognosis [9]. Clinical prediction models based on risk factors had been derived and validated for detecting aneurysmal subarachnoid haemorrhage [10]. Thus, we want to construct a clinical risk model using risk scores to evaluate the cancerization risk of OPMD. However, risk scores are based on population data. That is, the risk factors included in the model are applicable to most people not everyone. False-positive results could create fear in families until they are diagnosed as negative. Inevitably, false-negative cases would miss the chance for early diagnosis [11]. Therefore, the prediction of disease should be considered in combination with the actual situation and some detections.

Although histopathological examination is the gold standard for the diagnosis of OSCC, it is an invasive examination with poor reproducibility. Therefore, the minimally invasive or non-invasive early diagnosis techniques are more widely applied. Of them, autofluorescence is non-invasive, which can be easily operated. Nonetheless, its false-positive rate is high, and the accuracy rate is considerably affected by the experience of clinicians [12]. Besides, brush biopsy with DNA-image cytometry can diagnose OSCC at an early stage with high accuracy, but it still exists shortcomings [13].

This study aimed to build a clinical guide for the comprehensive assessment of the cancerization risk of OPMD patients by identifying the vital risk factors to construct a clinical risk model, combining with autofluorescence and brush biopsy with DNA-image cytometry.

Materials and methods

Patients

A total of 269 patients were collected from the Stomatological Hospital Affiliated to Nanjing Medical University (Nanjing, Jiangsu, China) from August 2016 to May 2018 for the cross-sectional analysis. Informed consent was obtained by each patient, and all the procedures were approved by the Ethical committee of Stomatological Hospital Affiliated to Nanjing Medical University.

Study inclusion criteria and protocol

Patients with the clinical and pathological diagnosis of OPMD (leukoplakia or oral lichen planus) or OSCC were enrolled in this study. The diagnostic criteria of OPMD or OSCC were determined based on the recommendations of WHO [14, 15]. Negative results of histopathological examination included no dysplasia, mild and moderate dysplasia of the epithelium, defined as negative group of histopathological examination (OPMD group); whereas the positive ones stood for epithelium with severe dysplasia or malignant cells, defined as positive histopathological group (OSCC group).

Baseline clinical data

Questionnaires were designed to gather patient information after they had signed the written informed consent, including gender, age, lesion site, lesion size, lesion character, local stimulus, smoking, drinking, system history and family history.

Clinical procedure

Each subject was treated successively based on the sequence of *Candidaalbicans* (*C. albicans*) test, autofluorescence and brush biopsy with DNA-image cytometry, prior to the biopsies of the suspected oral mucosal lesions.

Candida albicans

After patients rinsing, the lesions were repeatedly wiped with a sterile cotton swab gently. The collected samples were then cultured on the Sabouraud dextrose agar (Merck, Germany) for 48 h at 35 °C to detect the presence of Candida. Colonies of milky white rounded protrusions were picked out from the grown colonies, inoculated into Candida chromogenic medium, and cultured at 35 °C for 48 h. The green to emerald colony on the medium was *C. albicans*.

Autofluorescence

Conventional oral examination (COE) was performed in patients with oral mucosal lesions under incandescent operatory light and a provisional clinical diagnosis was also recorded, followed by autofluorescence examination using the VELscope (LED Medical Diagnostics Inc., Burnaby, Canada). Photos of all lesions were taken during COE and VELscope examination for future review. Positive diagnoses were indicated by fluorescence visualisation loss (VFL), whereas negative observations manifested as fluorescence visualisation retained (VFR).

Brush biopsy with DNA-image cytometry

The brushing site was selected according to the result of autofluorescence. If the result was positive, the exfoliated cells were brushed from the positive area; otherwise, the exfoliated cells of suspicious regions were blushed off according to COE.

The specific operations of the automated DNA-image cytometer (Wuhan Landing Medical Hing-Tech Co., Ltd., Wuhan, Hubei, China) referred to our previous study [16].

Finally, the negative diagnose indicated DNA euploid, whereas positive ones suggested that the cells containing DNA content > 5c, or the hyperplasia cells between 2 and 4c exceeding 10% of the total tested cells or appearing aneuploidy peak [16, 17].

Histopathological examination

Briefly, the formalin-fixed paraffin-embedded tissue sections were stained using hematoxylin and eosin (HE). The results were assessed by two experienced oral pathologists who were blinded to the findings of VELscope and DNA content and were not involved in the clinical arm of the study.

Statistical methods

Categorical data were compared using the Chi square test or Fisher's exact test. The clinical risk factors of canceriza-tion for OPMD were identified through univariate logistic regression analysis. Subsequently, the significant risk factors were evaluated by multivariate logistic regression analysis. The risk score of each element was determined according to the β coefficient [18]. Moreover, the cutoff value of the risk score was calculated using the ROC curve according to the sensitivity and specificity. Furthermore, the sensitivity, specificity, PPV and NPV were calculated for the clinical risk score ≥ cutoff value, autofluorescence and brush biopsy with DNA-image cytometry. At last, the ROC curves and DCA were drawn.

For univariate logistic regression analysis, $p < 0.10$ was considered as statistically significant. For the other tests, $p < 0.05$ was statistically significant. Statistical analysis was mainly performed using Stata version 13.0 (Stata Corp, College Station, TX, USA), and the DCA using R version 3.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The characteristics of OPMD ($n = 192$) and OSCC ($n = 77$), as well as the unadjusted odds ratio (OR) according to univariate analysis, are displayed in Table 1. As could be seen, age, lesion site, local stimulus, drinking, system history and *C. albicans* were the significant predictors at bivariate level.

There was a zero cell for lesion character, so its OR and 95% CI could not be estimated. Nonetheless, its Chi square value was 76.96, and p value was < 0.001, if the Chi square test was carried out. Therefore, the increase of 0.5 per cell was used for adjustment, and the calculated OR was 214.8, which was too large to be estimated reasonably, and was thereby excluded from the multivariate logistic regression model.

Typically, drinking was a three-category variable. If the first group was deemed as the reference group, the second group was meaningless, but the third group had shown a significant difference. Therefore, the first and second categories of alcohol consumption was combined in the multifactor model to produce a new variable drink (0 = never or ex-alcohol users, while 1 = current alcohol users).

Construction of the clinical risk model

Factors that were ultimately incorporated into the clinical risk model included gender, age, lesion site, local stimulus and alcohol drinking ($p < 0.05$). Then, a value (risk score) was assigned to each independent factor ($p < 0.05$) for the cancerization of OPMD according to the β coefficient in the multivariate logistic regression model (Table 2). Specifically, the variable with the lowest β coefficient (gender = 1.05) was denoted as 1, and the β coefficients of other variables were divided by 1.05 to reserve a decimal fraction. The corresponding reference groups were denoted as 0. Male = 0, female = 1; age < 55 = 0, age > 55 = 1.6; the lesion site at non-danger zone = 0, danger zone = 1; without local stimulus = 0, with = 1.4; and never or ex-alcohol users = 0, current alcohol users = 1.5. The clinical risk score of each patient represented the sum of scores of these five variables, which ranged from 0 to 1.6 points. The probability of the cancerization of OPMD corresponding to clinical risk score is shown in Table 3. The visualisation of clinical risk model is shown in Fig. 1. Clinical risk score = 1 × gender + 1.6 × age group + 1 × lesion site + 1.4 × local stimulus + 1.5 ×s drink.

Risk score analysis

A ROC curve was plotted using the five risk factors in the clinical risk score. Based on ROC curve analysis of the clinical risk model, the AUC was 0.83 (95% CI 0.77–0.88) (Fig. 2).

The optimal cutoff value was three by the maximum Youden index value. In addition, the sensitivity, specificity, PPV and NPV were 67.53%, 81.25%, 59.09% and 86.19%, respectively (Table 4).

The AUC implied the predictive ability of the model.

Outcomes of two adjunctive tests for clinical and histological assessment

All the 269 patients were examined with autofluorescence and DNA-image. Comparisons between two adjunctive tests and pathological diagnosis are presented in Table 5.

Table 1 Characteristics of OPMD and OSCC, as well as the unadjusted OR (odds ratio)

Characteristics	Value	All ($n=269$) N (%)	OPMD ($n=192$) N (%)	OSCC ($n=77$) N (%)	Unadjusted OR (95% CI)	Wald Z test	p
Gender							
Male	0	142 (52.8)	106 (55.2)	36 (46.8)	1.00		
Female	1	127 (47.2)	86 (44.8)	41 (53.2)	1.40 (0.83–2.39)	1.25	0.210
Age (years)							
< 55	0	116 (43.1)	103 (53.6)	13 (16.9)	1.00		
≥ 55	1	153 (56.9)	89 (46.4)	64 (83.1)	5.70 (2.94–11.03)	5.17	< 0.001
Lesion site							
Non-danger zone	0	193 (71.7)	150 (78.1)	43 (55.8)	1.00		
Danger zone[a]	1	76 (28.3)	42 (21.9)	34 (44.2)	2.82 (1.60–4.97)	3.60	< 0.001
Lesion character							
Homogeneous	0	112 (41.6)	112 (58.3)	0 (0)			
Heterogeneous	1	157 (58.4)	80 (41.7)	77 (100)	–	–	–
Local stimulus							
Without	0	180 (66.9)	150 (78.1)	30 (39.0)	1.00		
With	1	89 (33.1)	42 (21.9)	47 (61.0)	5.60 (3.16–9.91)	5.90	< 0.001
Smoking							
Never-smoked	0	193 (71.7)	140 (72.9)	53 (68.8)	1.00		
Ex-smokers	1	28 (10.4)	22 (11.5)	6 (7.8)	0.72 (0.28–1.87)	0.67	0.502
Current smokers	2	48 (17.8)	30 (15.6)	18 (23.4)	1.58 (0.82–3.08)	1.36	0.174
Drinking							
Never used	0	205 (76.2)	152 (79.2)	53 (68.8)	1.00		
Ex-users	1	23 (8.6)	18 (9.4)	5 (6.5)	0.80 (0.28–2.25)	0.43	0.668
Current users	2	41 (15.2)	22 (11.5)	19 (24.7)	2.48 (1.24–4.93)	2.58	0.010
System history							
Without	0	148 (55.0)	120 (62.5)	28 (36.4)	1.00		
With	1	121 (45.0)	72 (37.5)	49 (63.6)	2.85 (1.65–4.94)	3.75	< 0.001
Family history							
Without	0	263 (97.8)	188 (97.9)	75 (97.4)	1.00		
With	1	6 (2.2)	4 (2.1)	2 (2.6)	1.25 (0.22–6.99)	0.26	0.797
C. albicans							
Negative	0	216 (80.3)	162 (84.4)	54 (70.1)	1.00		
Positive	1	53 (19.7)	30 (15.6)	23 (29.9)	2.30 (1.23–4.29)	2.61	0.009

[a]Danger zone (the floor of mouth, the ventral tongue, the soft palate complex, and the inner triangle of the mouth corner)

Table 2 Multivariate logistic regression model for predictors of cancerization for OPMD and value for the predictors

Variable	Adjusted OR (95% CI)	Wald Z test	p	β coefficient	Value[a]
Female	2.86 (1.40–5.86)	2.87	0.004	1.05	1
Age ≥ 55	5.31 (2.54–11.12)	4.43	< 0.001	1.67	1.6
Danger zone	2.9 (1.47–5.72)	3.06	0.002	1.06	1
Local stimulus	4.38 (2.32–8.29)	4.54	< 0.001	1.48	1.4
Current alcohol users	4.9 (1.95–12.31)	3.38	0.001	1.59	1.5

[a]Risk score

Assessment of the cancerization risk in OPMD patients

The sensitivity, specificity, PPV, NPV, AUC and its 95% CI of the clinical risk score ≥ 3, autofluorescence and Brush biopsy with DNA-image cytometry were calculated based on the gold standard of histopathological examination (Table 4).

Table 3 Clinical risk score corresponding to the probability of the cancerization of OPMD

Score	*N*	*p*	Score	*N*	*p*
0	31	0.022	3.1	5	0.373
1	40	0.062	3.4	5	0.454
1.4	4	0.091	3.5	1	0.481
1.5	8	0.101	3.6	5	0.502
1.6	23	0.108	4	30	0.605
2	6	0.159	4.1	4	0.633
2.4	12	0.223	4.5	8	0.723
2.5	8	0.245	5	10	0.815
2.6	48	0.259	5.1	1	0.831
2.9	1	0.329	5.5	5	0.883
3	14	0.348			

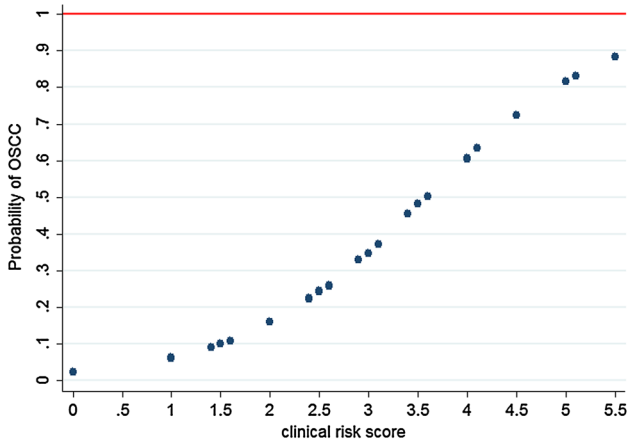

Fig. 1 Visualisation of clinical risk model

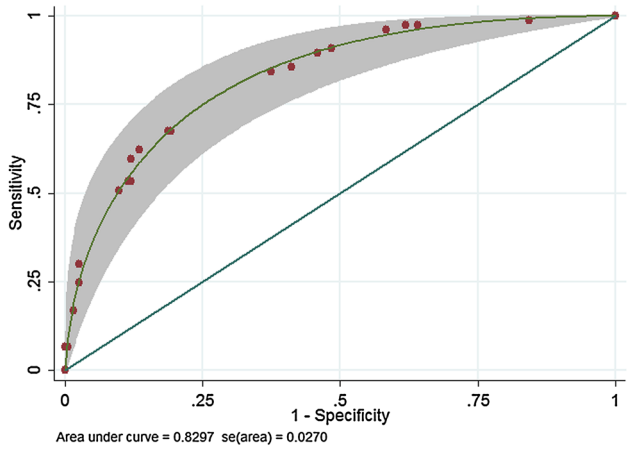

Fig. 2 The ROC curve of the clinical risk score for OSCC based on the five independent predictors in multivariate logistic regression

Moreover, the ROC curve was plotted to assess the diagnostic value of each prediction tool (Fig. 3). As shown in Table 4, the AUC of clinical risk score ≥ 3, autofluorescence and brush biopsy with DNA-image cytometry were 0.74, 0.77 and 0.92, respectively.

The DCA was performed to estimate the clinical usefulness and net benefit [19]. As could be observed in Fig. 4, "none" presented all the patients are positive of histopathology and "all" presented all are negative. The net benefit of all three predictive tools to predict OSCC was greater than "none" and "all", and brush biopsy with DNA-image cytometry was the greatest.

In the first line, the ROC curve was drawn based on the cumulative clinical risk score of various variables in Table 2. It was a continuous variable, and thus sensitivity, specificity, PPV and NPV were not provided under each grouping condition. Then, three scores were used as the boundary value segment to calculate its predictive ability.

Discussion

In our study, we analysed the risk factors that had a vital role in the cancerization of OPMD patients. The results of univariate regression analysis suggested that age, lesion site, lesion size, local stimulus, drinking, system history and *C. albicans* were the significant predictors. Gender, as a specific confounding factor, was also incorporated in the multivariate logistic regression analysis. Concretely, factors were that ultimately incorporated into the clinical risk model included gender, age, lesion site, local stimulus and alcohol drinking ($p < 0.01$). Notably, the clinical risk model was established as follows: clinical risk score = 1 × gender + 1.6 × age group + 1 × lesion site + 1.4 × local stimulus + 1.5 × drink; gender (0 = male, 1 = female), age group (0 ≤ 55, 1 ≥ 55), lesion site (0 = non-danger zone, 1 = danger zone), local stimulus (0 = without, 1 = with), and drink (0 = never or ex-alcohol users, 1 = current alcohol users). A higher cancer risk score stood for a higher cancerization risk of OPMD patients.

Our study showed that females have a higher risk of malignant transformation, which is consistent with that from the study by Warnakulasuriya et al. on gender and malignant transformation [20]. The cancerization risk of OPMD was higher in individuals aged ≥ 55 years. It is supported by many studies that lesions are likely to develop and progress in older individuals [21]. In addition, drinking, an independent risk factor, was found to play a significant role in our results, which was consistent with the findings of some previous studies [8, 22]. For non-smokers, alcohol consumption was consistently found to be associated with the risk of cancerization in oral precancerous lesions.

Table 4 Comparisons of the diagnostic reliability of the adjunctive tests

Tests	Se (%)	Sp (%)	PPV (%)	NPV (%)	AUC (95% CI)
Clinical risk score	–	–	–	–	0.83 (0.77–0.88)
Score ≥ 3	67.53	81.25	59.09	86.19	0.74 (0.68–0.80)
Autofluorescence	100.00	54.69	46.95	100.00	0.77 (0.74–0.81)
DNA content	93.51	90.10	79.12	97.19	0.92 (0.88–0.95)

Se sensitivity, *Sp* specificity

Table 5 Comparisons of results between autofluorescence or brush biopsy with DNA-image cytometry and histopathological examination

Pathological diagnosis	Cases (*n*)	Positive test results	
		Autofluorescence	DNA content[a]
Positive (OSCC)	77	77	72
Negative (OPMD)	192	87	19

[a]Brush biopsy with DNA-image cytometry

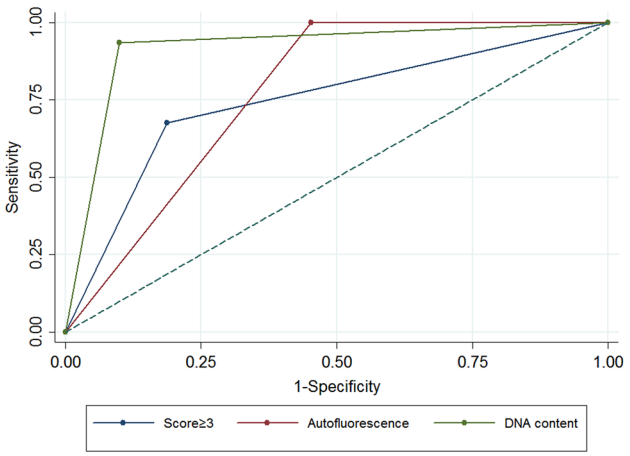

Fig. 3 The ROC curve for comparing the three prediction tools (clinical risk score of ≥ 3, autofluorescence and DNA content)

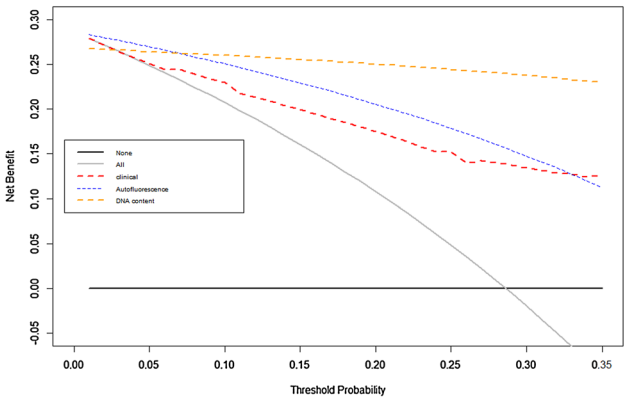

Fig. 4 The DCA for the clinical risk score, autofluorescence and brush content

Moreover, lesions occurring in the high-risk areas, such as the floor of mouth, the ventral tongue, the soft palate complex, and the inner triangle of the mouth corner, indicate an increased risk of malignant transformation [23, 24]. In our study, compared with other oral sites, the adjusted conversion risk in the danger zone was increased to 2.9. The local stimulation factors were registered in three groups, including sharp or broken tooth, bad prosthesis and severe periodontitis. And our results showed that the presence of local stimulus would remarkably increase the risk of cancerization in OPMD. Generally, oral cancer occurs mainly in locations that can be exposed to prosthetic or dental chronic mechanical irritation, especially in non-smokers without other risk factors [25]. A meta-analysis suggested that the use of removable dentures itself would increase the risk of developing oral cancer, and the risk would be much higher if they were the ill-fitting dentures [26].

In addition, our study showed that, *C. albicans* infection was statistically associated with the cancerization of OPMD upon single-factor analysis, with the OR of 2.30. But there was no statistical correlation in the multivariate logistic regression. Candida isolated from leukoplakic lesions may produce carcinogenic nitrosamines or acetaldehydes [27]. On this account, candida infections should be considered as the suspicious carcinogenic factors, which should be carefully examined at clinic.

The AUC implies the likelihood that a random test result will be ranked correctly as to the disease state. Generally, when the AUC was 0.7−0.9, indicating that the test has a certain predictive ability; while that more than 0.9, indicating a strong predictive ability. As described in the method, the brushing site of brush biopsy with DNA-image cytometry was selected according to the result of autofluorescence. In this study, the clinical risk score ≥ 3 (AUC = 0.74) and using autofluorescence alone (AUC = 0.77) both had a certain diagnostic values, while brush biopsy with DNA-image cytometry under the guidance of autofluorescence (AUC = 0.92) displayed a good diagnostic value. The DCA implies that net benefit is plotted against threshold probability. The decision curve of the model was compared with the two extreme cases where the

histopathological results were all positive and all negative. A model is of clinical utility if the net benefit of the model is greater than all patients are positive and all are negative [19]. The net benefit of three predictive tools to predict OSCC was greater than "none" and "all", suggesting that they all had clinical utility. In addition, brush biopsy with DNA-image cytometry was the most effective.

The clinical risk model can improve the recognition of risk factors and oral cancer in patients with OPMD, and contribute to the early prevention of the disease. The model in this study was based on the maximum Youden index value, and the optimal cutoff value was 3. In addition, the sensitivity, specificity, PPV and NPV of the clinical risk score ≥ 3 were 67.53%, 81.25%, 59.09% and 86.19%, respectively. In a population with high prevalence of OSCC, a higher NPV is preferable [28]. Based on the higher NPV (86.19%) of our model, we can assure OPMD patients with a lower risk score that he/she is unlikely to become OSCC and reduce their psychological burden. On the other hand, for screening models for fatal diseases such as oral cancer, sensitivity should be higher than specificity to reduce false negative at screening [29]. The specificity of our model was higher (0.813), while the sensitivity was medium (0.675). Therefore, patients with OSCC who are easily missed using this model alone should also rely on other detection method for targeted and relatively accurate evaluation.

As a non-invasive screening method, autofluorescence can be easily operated. In our study, the sensitivity, specificity, PPV and NPV of autofluorescence were 100.00%, 54.69%, 46.95% and 100.00%, respectively. Besides, a loss of fluorescence was detected in 100% of all OSCC, which showed the good ability to detect OSCC. In addition, autofluorescence has the ability to help locate the malignant lesions and find the right location for a biopsy [30]. However, the relatively low specificity of autofluorescence results in a large number of false-positive results, which were not clinically acceptable. Studies have shown that inflammation damage (such as ulcer and gingivitis), hyperemia and mucosal pigmentation also show fluorescence deficiency [31]. Thus, it is not appropriate to use autofluorescence alone as a clinical screening test, which accuracy is hugely affected by the experience of clinicians [12].

In this study, brush biopsy with DNA-image cytometry under the guidance of autofluorescence showed high sensitivity (93.51%) and high specificity (90.10%). In our previous experiment, the sensitivity and specificity of brush biopsy with DNA-image cytometry alone were 86.36% and 90% [16]. Compared with it, both the sensitivity and specificity were improved after the sequence application. Autofluorescence is less specific; nonetheless, it is highly sensitive. In clinical application. Moreover, with quite efficient localisation of lesions, autofluorescence was of great guiding significance for the brushing site of brush biopsy with DNA-image cytometry.

In conclusion, it remains a clinically difficult problem to determine the timing and location of histopathological examination for OPMD. Under such circumstances, the clinical risk model can be used to improve the recognition of risk factors in patients with OPMD. The risk of carcinogenesis will be preliminarily assessed according to the score of OPMD patients, and health education related to risk factors can be conducted for them. Furthermore, the sequential use of autofluorescence and brush biopsy with DNA-image cytometry can assess the risk of carcinogenesis accurately in patients with OPMD to determine whether histopathological examination or other intervention is required. In addition, histopathological examination can be temporarily avoided for low-risk OPMD, and the sequential monitoring method can be adopted for chronic disease management, with an aim to reduce the psychological and economic burden on patients.

We followed up 46 patients for up to 13–27 months after the first evaluation. Among them were 40 patients with OPMD who were stable. There were no cases of carcinogenesis after clinical risk model-related oral health education as well as drug or photodynamic therapy. However, OPMD has a specific risk of carcinogenesis, in which the average carcinogenesis time for oral leukoplakia is 5.2 years, and the cancerization rate increases with the prolongation of follow-up time [32]. As clinicians, it is necessary for us to carry out regular examinations on patients, and even shorten the follow-up intervals for high-risk patients to identify hidden lesions at an early stage. The remaining six cases were that of OSCC patients; one of them had died, and the other five had not yet experienced any recurrence after the surgical resection. Despite substantial progress in diagnosis and management, there has not been any significant improvement on the 5-year survival rate of OSCC patients in recent decades [33]. We are applying the non-invasive methods on the patients after the OSCC operation to explore the significance of monitoring the recurrence of the disease and achieve the purpose of lifelong management.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval This study was carried out following the guidelines of the Declaration of Helsinki and was approved by the Ethical Committee of Stomatological Hospital Affiliated to Nanjing Medical University (no. PJ2016-034–001).

Informed consent Informed consent was obtained by each patient. Moreover, patient information was anonymised and de-identified before analysis.

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