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Prognostic impact of muscle invasion in buccal mucosa squamous cell carcinoma

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Abstract

Objective: The objective of the study was to assess the prognostic value of muscle invasion (MI), a key histopathological feature of tumor aggressiveness, and construct a superior prognostic prediction model combining the current TNM staging system.

Materials and Methods: MI was analyzed in the whole-slide images from a total of 301 patients with primary buccal mucosa squamous cell carcinoma (BMSCC). Survival times of patients with/without MI were evaluated by Kaplan–Meier analysis. MI was further combined with the TNM staging system to explore its predictive value for prognosis. Moreover, 204 cases of head and neck carcinoma from the TCGA database were included.

Results: MI positive rate reached to 76% (229/301) in patients with BMSCC. MI was associated with poor overall survival ($p = 0.012$) and disease-free survival ($p = 0.022$). The novel system (TNM staging combined with MI) revealed strong predictive performance, with the largest area under the curve (OS: $p < 0.001$, DFS: $p < 0.004$). MI and the established classification system were also had good predictive ability in the TCGA cohort.

Conclusions: MI is an independent predictor of poor prognosis of BMSCC. The inclusion of MI in prediction system can augment the risk stratification of patients with oral squamous cell carcinoma and may assist in the clinical decision-making process.

KEYWORDS
buccal mucosa squamous cell carcinoma, muscle invasion, pathology, prognosis, TNM staging system

Yan Wu and Shuai Wang contributed equally to this work.

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a characteristic locally aggressive tumor, accounting for more than 90% of all oral cancers, and is a major global public health problem (Johnson et al., 2011). The incidence and mortality rates of OSCC have been increasing in recent decades (Sasahira & Kirita, 2018; Siegel et al., 2022). Even with advances in diagnostic and therapeutic techniques, there are still more than 300,000 new cases of and 150,000 deaths from OSCC each year (Sung et al., 2021). Buccal mucosal squamous cell carcinoma (BMSCC) accounts for about 10% of OSCC, and this number is higher in Southeast Asian countries (Bobdey, Sathwara, et al., 2018).

As a biologically distinct entity, BMSCC has a high recurrence rate and high ability to invade. This is closely related to the unique anatomical structure of the buccal region, which is basically ordered from the mucosal layer to the skin layer basically including submucosal fascia, fatty tissue, muscle tissue, fatty tissue, and subcutaneous fascia. BMSCC exhibits a different biological behavior after breaking through the basal layer, with some tumors being blocked by submucosal adipose tissue and then spreading parallel to the surrounding area, while others continue to invade deeper into the tissue and break through muscle tissue or even superficial skin. However, it is unclear whether MI by BMSCC affects patient prognosis.

With the increasing understanding of tumor biological behavior, the important role of the tumor microenvironment in oncogenesis, progression, invasion, and metastasis has been gradually realized (Chen et al., 2021; Hinshaw & Shevde, 2019). As a non-tumor cell component of tumor microenvironment (TME), muscle tissue has been well demonstrated to be involved in tumor progression. In bladder, rectal and endometrial cancers, the endothelial and fascicular spaces of muscle cells, can serve as a channel for tumor invasion (Harryman et al., 2021; Magers et al., 2019; Wang et al., 2021). MI has been shown to predict higher occult lymph node metastasis in OSCC and has a higher positive predictive value for local tumor recurrence than depth of invasion (DOI) (Chandler et al., 2011). However, the clear definition of MI and the impact of its occurrence on the prognosis of BMSCC patients remain unclear. Here, combined with the literature and guidance from pathologists, we define MI as the isolation of tumor cells from the primary tumor mass and their active movement and invasion into the surrounding muscle tissue (Beunk et al., 2019; Bobdey et al., 2018).

In this work, we analyzed HE-stained tissue sections of 301 patients with primary BMSCC to clarify whether MI is a prognostic predictor for cancer patients. We also explored how to combine MI with conventional tumor–node–metastasis (TNM) staging to better predict the prognosis of patients with BMSCC.

2 | MATERIALS AND METHODS

2.1 | Study cohort

Patients with primary BMSCC who underwent resection at Nanjing Stomatology Hospital between 2013 and 2018 were enrolled in this study. Clinical and pathological data were obtained from files at the Department of Pathology and the Histology Repository of Nanjing Stomatology Hospital. The criteria for inclusion were as follows: (1) patients with BMSCC; (2) no preoperative chemotherapy, radiation therapy; (3) follow-up data of at least 3 years for survivors. The exclusion criteria were as follows: (1) patients with the disease involving other anatomical sites; (2) incomplete case or follow-up data; (3) slides with poor staining quality or slices with incomplete deep margins. Participant information covered major clinical and demographic features and pathological features, including T stage, N stage, histological differentiation, clinical stage, DOI, pattern of invasion (POI), worst pattern of invasion (WPOI), perineural invasion (PNI), tumor budding (TB), tumor–stroma ratio (TSR), tumor-infiltrating lymphocytes (TIL), and Ki-67 expression levels. In addition, we also obtained data from the TCGA database for 499 patients diagnosed with head and neck squamous cell carcinoma (HNSCC). However, because of the low quality of staining of some slides, only 204 cases were used as an independent cohort to validate our results. All methods used in this study were approved by the ethics committee of Nanjing Stomatology Hospital (January 18, 2022, NJSH-2022NL-016), and this study was conducted in accordance with the Declaration of Helsinki. As this was a retrospective study, the requirement for informed consent was waived.

2.2 | Histopathological evaluation

HE-stained slides obtained from the Pathology Department of Nanjing Stomatology Hospital were scanned into high-resolution (0.14 μm/pixel) digital images at 40× magnification using a 3DHistech Pannoramic MIDI II scanner (3DHISTECH Ltd.). The digital HE-stained slides were also reviewed by CaseViewer 2.4 for Windows (3DHISTECH Ltd.), a digital application for evaluating microscopy images. Specific procedures have been previously published (Wang et al., 2022). After digitization of the tissue slides was completed, histological evaluation was performed independently by two experienced pathologists (X.H. and L.Z). We defined the invasion of tumor cells into the surrounding muscle tissue as positive for muscle invasion (MI+), when the pathology showed direct contact between tumor and muscle cells. Tumor cell emboli were noted within muscle tissue. On the contrary, it was defined as negative for muscle invasion (MI−) once tumor cells have not invaded the surrounding muscle tissue, and the pathology showed that tumor cells did not have direct contact with muscle cells (Figure 1, Figure S1).

2.3 | Statistical analysis

The following analytical tools were used for this study: SPSS 26.0 (IBM Corporation) and GraphPad Prism 8.0 (GraphPad Software, Inc.). Association of MI with other parameters was analyzed descriptive statistical methods and using chi-square. Kaplan–Meier survival analysis was applied for the analysis of the survival curves. Univariate Cox regression analysis was used to estimate the association

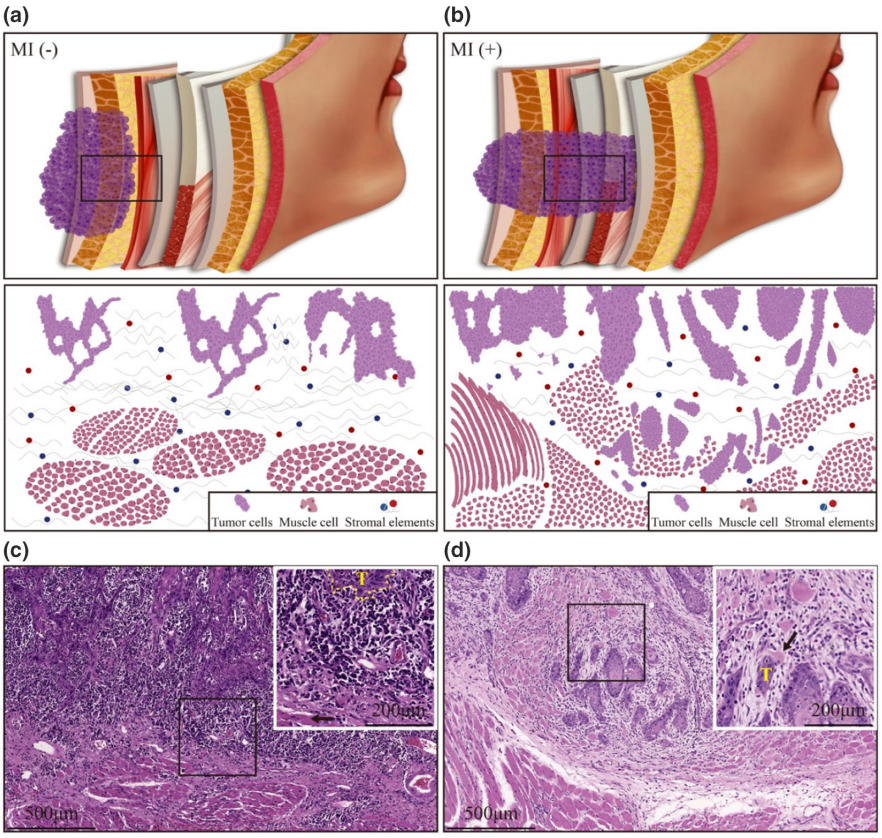

FIGURE 1 Schematic image of muscle invasion of BMSCC. (a) MI (−): The deepest edge of the cancerous tissue does not make direct contact with the muscle tissue. (b) MI (+): Tumor clusters are in direct contact and interlaced with muscle cells. As shown in the lower right corner, there are muscle cells, tumor cells and stromal elements such as inflammatory cell in the figure. (c) Representative HE-stained images of MI (−), the yellow dashed line in the upper right corner of the figure indicates the tumor area. (d) Representative HE-stained images of MI (+). T: tumor cell. The black arrow indicates the tumor. +, positive; −, Negative.

between variables and survival outcomes (OS, DFS). In addition, the predictive power of different combinations of values was assessed by combining MI and other pathological parameters by receiver operating characteristic curve (ROC) analysis and area under the ROC curve (AUC) values. A *p*-value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographic and clinicopathological variables

A total of 301 patients with BMSCC were included in this study. The histological slides were also reviewed and evaluated for histopathological parameters. All these patients were reassessed and restaged according to the 8th edition American Joint Committee on Cancer (AJCC) staging system. The general characteristics of the study patients are shown in Table 1. The majority of the patients were over 60 years of age ($n = 227$; 75.4%), with a median age at diagnosis of 65 years. The ratio of males (165 [54.8%]) to females (136 [45.2%]) was 1.21:1. Overall, 43.52% (131/301) of patients were smokers. Of the 301 cases, 44 (14.33%) tumors were stage pT1, 143 (47.51%) were stage pT2, 53 (17.61%) were stage pT3, and 61 (20.27%) were stage pT4. Lymph node metastasis occurred in 115 tumors (38.21%) and did not occur in 186 tumors (61.78%). A total of 129 patients (42.86%) were in clinical stage I-II, and 172 patients (57.14%) were in stage III-IV. In addition, 64 (21.26%) were highly differentiated, and 237 (78.74%) were moderately to poorly differentiated. 104 (34.55%) patients with depth of invasion (DOI) less than 5 mm, and 197 (65.45%) patients greater than 5 mm. As for the pattern of invasion (POI), 225 (74.75%) patients were POI 1–3, 76 (25.25%) patients were POI 4–5; regarding the worst pattern of invasion (WPOI), 203 (67.44%) patients were 1–3, 98 (32.56%) patients were 4–5. Perineural invasion (PNI) occurred in 98 tumors (32.56%) and did not occur in 203 tumors (67.44%). A total of 153 (50.83%) cases had high Ki67 expression, and 148 (49.17%) had low Ki67 expression.

3.2 | Association between MI and clinicopathological features in all patients

Clinicopathological data showed that the presence or absence of MI was positively correlated with pathological T stage ($X^2 = 9.898$, $p = 0.019$), pathological N stage ($X^2 = 10.241$, $p = 0.001$), TNM stage ($X^2 = 9.256$, $p = 0.002$), degree of differentiation ($X^2 = 24.455$, $p = 0.001$), DOI ($X^2 = 37.723$, $p = 0.001$), POI ($X^2 = 3.942$, $p = 0.047$), WPOI ($X^2 = 9.640$, $p = 0.002$), PNI ($X^2 = 10.898$, $p = 0.001$), and high or low expression of Ki67 ($X^2 = 5.400$, $p = 0.020$), but gender ($X^2 = 0.173$, $p = 0.678$), age ($X^2 = 1.072$, $p = 0.301$), and smoking ($X^2 = 0.527$, $p = 0.468$) did not correlate. Specifically, for patients without MI, 25.0% were in T1 stage and 12.5% in T4 stage; while for patients with MI, the proportion of T1 stage decreased to 11.4%, and T4 stage increased to 22.7%. The proportion of T2 and T3 did not change significantly. For the N status, 77.8% of

TABLE 1 Association between MI and clinicopathological features in all patients.

Characteristics	*N* (=301)	Muscle invasion		OR	95CI	χ^2	*p*
		–	+				
Gender							
Female	136	31 (43.1)	105 (45.9)	1.120	0.657–1.910	0.173	0.678
Male	165	41 (56.9)	124 (54.1)				
Age (years)							
<60	74	21 (29.2)	53 (23.1)	1.367	0.755–2.476	1.072	0.301
≥60	227	51 (70.8)	176 (76.9)				
Smoking							
No	170	38 (52.8)	132 (57.6)	0.821	0.483–1.398	0.527	0.468
Yes	131	34 (47.2)	97 (42.4)				
T							
1–2	187	51 (70.8)	136 (59.4)	1.661	0.937–2.944	3.049	0.081
3–4	114	21 (29.2)	93 (40.6)				
N							
–	186	56 (77.8)	130 (56.8)	2.665	1.442–4.925	10.241	***0.001***
+	115	16 (22.2)	99 (43.2)				
Clinical stage							
1–2	129	42 (58.3)	87 (38.0)	2.285	1.333–3.918	9.256	***0.002***
3–4	172	30 (41.7)	142 (62.0)				
Differentiation							
Well	64	30 (41.7)	34 (14.8)	4.218	2.326–7.649	24.455	***0.001***
Moderately + Poorly	237	42 (58.3)	195 (85.2)				
DOI							
<5 mm	104	42 (77.8)	62 (25.1)	9.852	4.348–22.324	37.723	***0.001***
≥5 mm	197	12 (22.2)	185 (74.9)				
POI							
1–3	225	47 (87.0)	178 (72.1)	2.656	0.983–7.178	3.942	***0.047***
4–5	76	7 (13.0)	69 (27.9)				
WPOI							
1–3	98	29 (53.7)	69 (27.9)	2.987	1.469–6.071	9.640	***0.002***
4–5	203	25 (46.3)	178 (72.1)				
PNI							
–	203	49 (90.7)	154 (62.3)	5.282	1.797–15.521	10.898	***0.001***
+	98	5 (9.3)	93 (37.7)				
Ki-67							
Low (<40%)	148	44 (61.1)	104 (45.4)	1.889	1.100–3.243	5.400	***0.020***
High (≥40%)	153	28 (38.9)	125 (54.6)				
TB							
BD1 (0–4)	153	48 (66.7)	105 (45.9)	2.362	1.356–4.113	9.496	***0.002***
BD2-3 (≥5)	148	24 (33.3)	124 (54.1)				
TSR							
Low (<50%)	154	48 (66.7)	106 (46.3)	2.321	1.333–4.041	9.104	***0.003***
High (≥50%)	147	24 (33.3)	123 (53.7)				
TIL							
Grade 1–2 (<50%)	227	46 (63.9)	181 (79)	0.556	0.259–1.193	2.306	0.129
Grade 3 (≥50%)	74	26 (36.1)	48 (21)				

Note: Significant results are highlighted in bold and italics, significance level $p < 0.05$.

Abbreviations: CI, confidence interval; DOI, depth of invasion; MI, muscle invasion; MI+, MI positive; MI–, MI negative; OR, odd ratio; PNI, perineural invasion; POI, pattern of invasion; TB, tumor budding; TIL, tumor-infiltrating lymphocytes; TSR, tumor–stroma ratio; WPOI, worst pattern of invasion.

MI- patients without lymph node metastasis and 22.2% with lymph node metastasis; among MI+ patients, the proportion of patients without lymph node metastasis decreased to 56.8%, while those with lymph node metastases increased to 43.2%. In terms of other histopathological features, such as clinical stage, similar trends were observed.

3.3 | The prognostic value of MI

Patients with BMSCC were divided into two groups according to the occurrence of MI: the MI+ group and the MI− group. Kaplan–Meier analysis revealed that MI status strongly differentiated two groups with different prognoses (Figure 2). MI+ patients had worse OS ($p<0.0001$, Figure 2a), DFS ($p=0.0037$, Figure 2b), MFS ($p=0.0048$, Figure 2c), and RFS ($p=0.0234$, Figure 2d) than MI− patients. Moreover, TCGA data also confirmed a significant difference in prognosis between the MI− and MI+ groups (Figure 2e,f). Specifically, OS ($p=0.0001$) and DFS ($p=0.0114$) were significantly worse in MI+ patients than in MI− HNSCC patients. The results of the Cox analysis of OS and DFS are presented in Table 2. Univariate analysis showed that prognostic factors such as age, sex, and T stage were not associated with significant differences in OS. However, the presence or absence of smoking ($p=0.003$), lymph node status ($p=0.001$), total stage ($p=0.008$), cellular differentiation (good vs. moderate to poor, $p=0.001$), DOI ($p=0.011$), POI ($p=0.001$), WPOI ($p=0.007$), PNI ($p=0.027$), and MI ($p=0.001$) were significant determinants of OS. In addition, T stage ($p=0.015$), total stage ($p=0.017$), cellular differentiation (good vs. moderate to poor, $p=0.024$), DOI ($p=0.020$), Ki-67 level ($p=0.018$) and MI ($p=0.012$) were significant determinants of DFS. The results of univariate analyses indicated that MI was a prognostic factor for OS ($p=0.001$) and DFS ($p=0.012$). Compared with the MI− group, patients in the MI+ group had a 972.5% increased risk of death and a 527.0% increased risk of disease. TCGA data also showed that the categorical variable MI was significant for OS, and MI+ was associated with poorer OS (HR, 4.747; 95% CI, 2.464–9.145; $p=0.001$; Table S1).

3.4 | Predictive value of MI combined with parameters of TNM staging system

Based on the results of the chi-square test and Cox regression test described above, the following three parameters were selected to create a joint model of MI: T classification, N classification, and TNM stage. The new factors generated were MI-T, MI-N, and MI-S (Figure 3a,d,g). Each new factor is divided into three stages: 0, 1, and 2. The T classification was divided into two groups, T1-2 and T3-4. The T1-2 group was combined with MI-, while MI-T was 0. Either the T1-2 group was combined with MI+ or T3-4 was combined with MI−, MI-T was 1. T3-4 was combined with MI+, then MI-T was 2. The joint model for the other parameters was similar to the joint model for the T classification. The N classification was divided into two groups: no lymph node metastasis (N−) and developed lymph node metastasis (N+). The N− group was combined with MI−, while MI-N was 0. Either the N+ group was combined with MI− or N− was combined with MI+, MI-N was 1. N+ was combined with MI+, MI-N was 2. TNM stage was divided into two groups: stage 1–2 and stage 3–4. The indicator was combined with MI to form MI-S. Similarly, the stage 1–2 group was combined with MI−, MI-S is 0. The stage 1–2 group was combined with MI+, or the stage 3–4 group was combined with MI−, MI-S is 1. The stage 3–4 group was combined with MI+, MI-S is 2. A detailed summary of the joint staging system is reported in Figure 3a. The Kaplan–Meier survival curves for the three models are shown in Figure 3. The data showed that OS (all $p<0.05$, Figure 3b,e,h) and DFS (all $p<0.05$, Figure 3c,f,i) were worse for patients staged 2 after reclassification based on MI in the combined T, N, and TNM stage systems.

ROC analysis was used to further assess the predictive value of the combined model. Regarding OS (Figure 4a–c), the combined parameters were significantly different compared with the original parameters of T, N, and TNM stage (all $p<0.05$) (Table S2). The AUC for all the combined parameters was greater than that of the original parameters. A similar result was found for DFS (Figure 4d–f), where the joint model yielded better predictions (all $p<0.05$) (Table S2). The results of this analysis show that the joint model has better potential diagnostic value.

To validate the joint model, patients in the TCGA cohort were also reclassified. The joint model showed significant differences in OS and DFS by the log-rank test and Kaplan–Meier survival curves (all $p<0.05$; Figure S2). The results also indicated that the joint model was a robust predictive staging model with the largest area under the curve (Figure S3, Table S3). As above, we have demonstrated that the use of joint parameters has a better identification and predictive power of prognosis prediction.

4 | DISCUSSION

The surrounding environment of BMSCC is characterized by distinct structure and rich tissue types. Unlike tongue cancer, the surrounding environment is made up almost entirely of muscle components. It is also different from gingival cancer, where the epithelium is adjacent to bone tissue. In buccal cancer, the cancer cells invade and spread in a relatively "non-special" microenvironment, so they are selected as the object of study.

Our study provides the first of its kind clear definition of MI. Moreover, we have shown for the first time that MI is a prognostic factor for OS and DFS. Furthermore, we combined MI with the TNM staging system to explain its predictive value of prognosis. The new prognostic model showed better prognostic predictive performance than the conventional AJCC staging system (8th edition).

Currently, several pathological parameters, such as DOI, WPOI, TB, TIL, and TSR, are widely considered potential biomarkers of OSCC (Morais et al., 2023). DOI has been incorporated into

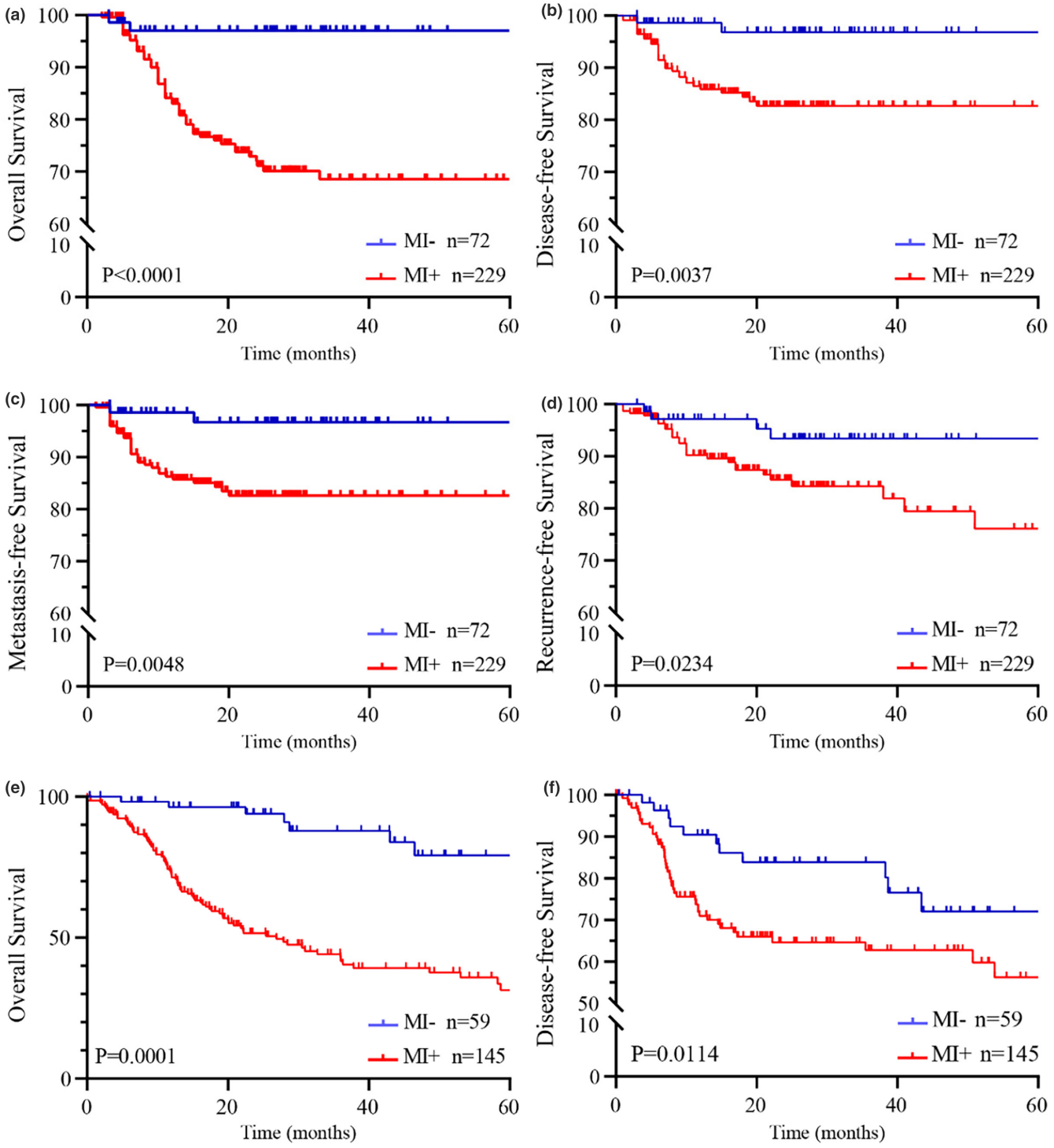

FIGURE 2 Evaluation of the prognostic value of MI status. (a–d) Kaplan–Meier analysis of 301 patients with BMSCC from our hospital for overall survival (a), disease-specific survival (b), metastasis-free survival (c), and recurrence-free survival (d). (e, f) Survival analysis in the OSCC patient cohort from TCGA. Survival curves were calculated using the Kaplan–Meier method and compared using the log-rank test.

the staging system of OSCC. WPOI and TB are two particularly important parameters for determining the risk of lymph node metastasis in OSCC. Furthermore, the prognostic value of TSR in OSCC and other solid tumors has been demonstrated. The indicator of MI is very common in pathology reports, and clinicians and pathologists regard it as one of the markers of poor prognosis. However, unlike prognostic parameters such as DOI and WPOI, there is currently no clear definition of MI, and there are few reports analyzing its prognostic value (Chaitra et al., 2020; Lewis, 2017; Patel et al., 2021). In the present study, MI was defined as the active movement of tumor cells into surrounding muscle tissue and pathology showed direct contact between tumor cells and muscle

TABLE 2 Cox regression analyses of OS and DFS.

	Overall survival			Disease-free survival		
Variables	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Gender						
Male	1	0.815–2.211	0.247	1	0.459–1.750	0.748
Female	1.343			0.896		
Age						
<60	1	0.856–3.156	0.135	1	0.717–4.165	0.223
≥60	1.644			1.729		
Smoking						
No	1	0.229–0.734	***0.003***	1	0.366–1.448	0.366
Yes	0.409			0.728		
T						
1–2	1	0.954–1.599	0.109	1	1.086–2.123	***0.015***
3–4	1.235			1.518		
N						
–	1	1.575–4.44	***0.001***	1	0.927–3.503	0.083
+	2.645			1.802		
Clinical stage						
1/2	1	1.103–1.943	***0.008***	1	1.089–2.332	***0.017***
3/4	1.464			1.593		
Differentiation						
Well	1	1.573–5.375	***0.001***	1	1.137–6.011	***0.024***
Moderately + Poor	2.908			2.615		
DOI						
<5 mm	1	1.260–5.889	***0.011***	1	1.223–10.243	***0.020***
≥5 mm	2.724			3.539		
POI						
1–3	1	1.751–6.001	***0.001***	1	0.885–4.447	0.096
4–5	3.241			1.984		
WPOI						
1–3	1	1.333–6.248	***0.007***	1	0.732–4.117	0.211
4–5	2.886			1.736		
PNI						
–	1	1.084–3.691	***0.027***	1	0.540–2.688	0.649
+	2.0			1.205		
Ki-67						
Low (<40%)	1	0.964–2.663	0.069	1	1.157–4.825	***0.018***
High (≥40%)	1.602			2.362		
TB						
BD1 (0–4)	1	1.112–2.261	***0.011***	1	1.016–2.472	***0.042***
BD2-3 (≥5)	1.586			1.585		
TSR						
Low (<50%)	1	0.904–3.526	0.095	1	0.409–2.107	0.859
High (≥50%)	1.786			0.928		
TIL						
Grade 1–2 (<50%)	1	0.414–1.066	0.090	1	0.434–1.278	0.285

(Continues)

TABLE 2 (Continued)

Variables	Overall survival			Disease-free survival		
	HR	95% CI	p	HR	95% CI	p
Grade 3 (≥50%)	0.665			0.745		
MI						
–	1	2.613–44.022	***0.001***	1	1.503–26.159	***0.012***
+	10.725			6.27		

Note: Significant results are highlighted in bold and italics, significance level $p < 0.05$.

Abbreviations: CI, confidence interval; DFS, disease-free survival; DOI, depth of invasion; HR, hazard ratio; MI, muscle invasion; MI+, MI positive; MI−, MI negative; OS, overall survival; PNI, perineural invasion; POI, pattern of invasion; TB, tumor budding; TIL, tumor-infiltrating lymphocytes; TSR, tumor–stroma ratio; WPOI, worst pattern of invasion.

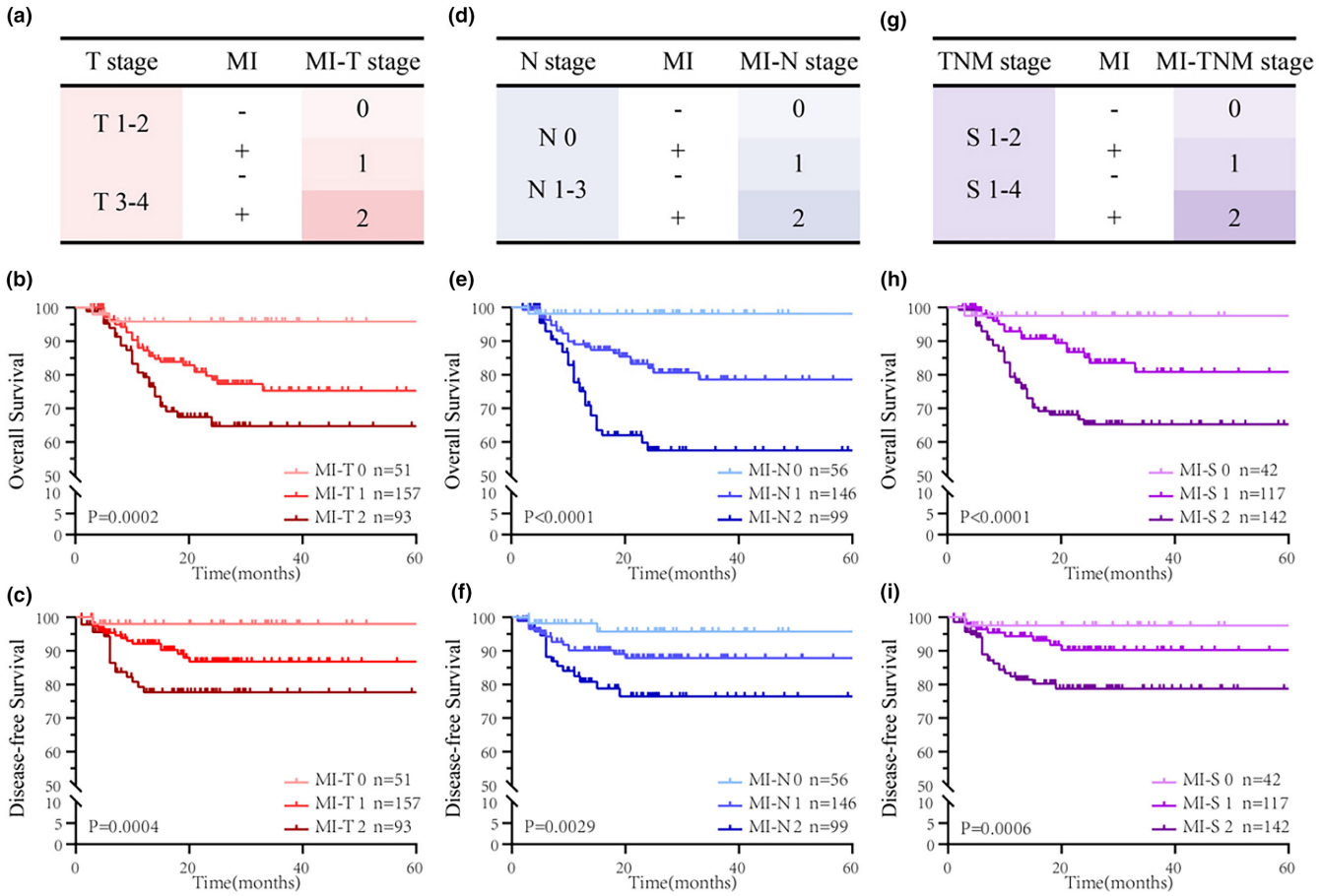

FIGURE 3 Assessment of the prognostic value of MI and TNM staging combinations. (a–c) The combination of MI and T staging and the KM survival curve of the new staging system. (d–f) The combination of MI and N staging and the KM survival curve of the new staging system. (g–i) The combination of MI and TNM staging and the KM survival curve of the new staging system.

cells. There is a critical state that manifests as the tumor have invaded the epimysium structure of muscle tissue but has no contact with muscle cells. Whether such condition classified as MI+ or MI− is a point of debate. However, due to the small proportion of this situation, whether it is positive or negative has little impact on the statistical results. In our current research, the critical state is classified as MI− because direct contact between tumor and muscle cannot be observed on the section. The role of MI in predicting the prognosis of patients with BMSCC was demonstrated by including 301 patients in the prognostic analysis. The TCGA sample cohort further validated our hypothesis. The results showed that MI combined with the TNM staging system is more effective in predicting prognosis, which can provide a more ideal solution for patient treatment and prognostic assessment and compensate for the deficiency of the current TNM classification (He et al., 2021; Huang & O'Sullivan, 2017). In addition, MI is easy to be observed in pathological sections and easy to be detected in biopsy. Therefore, although many other pathological parameters are related to

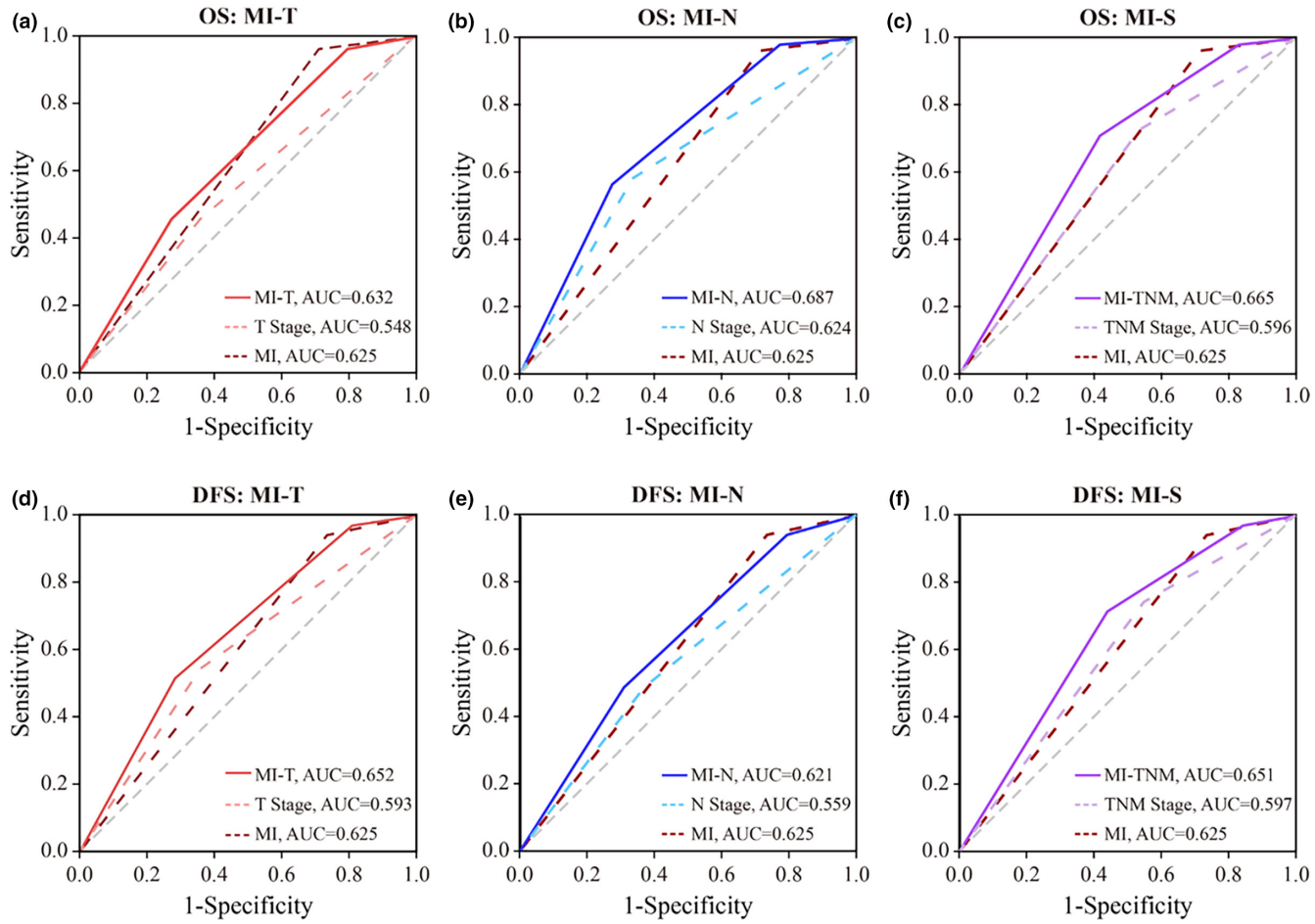

FIGURE 4 Assessment of the prognostic value of MI and other parameter combinations. Classification of MI and other parameters combined (a). Kaplan–Meier analysis of the 301 BMSCC patient cohorts from our hospital for overall survival (b–d) and disease-specific survival (e–g). (b, e) Combination of MI and T stage; (c, f) Combination of MI and N stages; (d, g) Combination of MI and clinical stage.

poor prognosis, the clinical application of MI is superior to those inconvenient to observe, such as DOI.

Our results demonstrate an association between MI and several classic pathological parameters in OSCC. The strong correlation between muscle invasion status and each pathological parameter may be due to the fact that muscle invasion status results from a combination of various pathological factors. MI, along with other pathological parameters, such as lymph node metastasis, poor differentiation, deeper infiltration depth, worse WPOI, positive PNI status, high Ki67 expression, higher TSR, and TB, can predict clinical outcomes. This indicates that muscle invasion status reflects cell proliferation, migration, and invasion within the tumor (Beunk et al., 2019; Bobdey et al., 2018). Combined parameters, rather than single parameters, are the ideal tools for OSCC prognosis (Morais et al., 2023). Our findings also provide evidence that combined parameters resulting from the integration of muscle invasion with T, N, and TNM stage offer enhanced predictability of prognosis.

In malignant tumors, such as bladder cancer and prostate cancer, tumor cell invasion of smooth muscle is considered a poor prognostic indicator (Harryman et al., 2021; Knowles & Hurst, 2015; Magers et al., 2019; Tilki et al., 2010; Wang et al., 2021). For example, in prostate cancer, cancers confined to the organ are classified as T1 and T2, and when the smooth muscle layer is disrupted, they are classified as T3 and T4, requiring more aggressive treatment (Harryman et al., 2021). For bladder cancer, the treatment strategy for non-muscle-invasive bladder cancer is significantly different from that for muscle-invasive bladder cancer according to the latest treatment guidelines (Babjuk et al., 2022; Witjes et al., 2021). However, there are few studies on tumor cell invasion of skeletal muscle (Cheung et al., 2013; Surov et al., 2010). Jie et al. (2021) used MI as a routine indicator of poor outcome by default (Mendenhall et al., 2009). Chandler et al. (2011) suggested that MI could be used as a proxy for depth of invasion to assess the risk of lymph node involvement and local recurrence (Newman et al., 2021). These findings are consistent with our findings. Tumor cell invasion of skeletal muscle appears to have the same role as tumor cell invasion of smooth muscle in affecting cancer prognosis. However, a more in-depth study of MI in skeletal muscle is necessary and relevant due to the structural differences between smooth muscle and skeletal muscle (Beunk et al., 2019; Calabrese et al., 2020).

Our study had a large sample size, the reliability of the results was high, and the TCGA data were also assessed for validation. In

addition, the study focused on skeletal muscle-related MI in oral squamous cell carcinoma, which has been neglected in previous studies. Our study fills this gap. Finally, our findings can help guide the management of oral cancer and provide a basis for other studies that directly investigate MI. Our study still has several limitations. First, our present study was conducted as a single-center study, and thus single-center effects cannot be excluded. Therefore, multicenter study incorporating more cases from different hospitals can be better elucidated can better clarify the diagnostic value of MI. Second, this observational retrospective study is subject to the limitations of any retrospective study. Prospective cohort study is needed to verify the diagnostic value of MI in the future. Finally, the sub-classification and molecular mechanism of MI still needs further research.

5 | CONCLUSIONS

In conclusion, we defined the concept of MI in OSCC, and the role of MI in the prognosis prediction of OSCC patients was validated by two patient cohorts using retrospective and in silico data. We further incorporated MI into the TNM staging system, increasing the risk stratification of the 8th edition AJCC staging system. As one of the promising prognostic parameters in the pathological diagnostic process, we hope that there will be larger sample studies or prospective cohort study to verify its prognostic predictive performance in the future. Once the diagnostic value of MI is verified in the setting of prospective multicenter trials, MI can be potentially used in daily diagnostic work.

AUTHOR CONTRIBUTIONS

Yan Wu: Conceptualization; validation; writing – original draft. **Shuai Wang:** Methodology; validation; writing – original draft. **Weixian Zhang:** Software; validation. **Feng Zhu:** Formal analysis. **Lei Zhang:** Investigation. **Sheng Chen:** Resources. **Chuanjin Ye:** Resources. **Yawei Sun:** Data curation. **Xiaofeng Huang:** Visualization; funding acquisition. **Antonio Celentano:** Writing – review and editing; supervision; project administration. **Yanhong Ni:** Conceptualization; writing – review and editing; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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