Virchows Archiv (2021) 479:109–119
https://doi.org/10.1007/s00428-020-03008-y

ORIGINAL ARTICLE

Biopsy pattern of invasion type to determine the surgical approach in early-stage oral squamous cell carcinoma

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Received: 23 August 2020 / Revised: 3 December 2020 / Accepted: 23 December 2020 / Published online: 13 January 2021

Abstract

Depth of invasion (DOI) and pattern of invasion (POI) indicate tumor invasiveness of oral squamous cell carcinoma (OSCC). However, preoperative DOI evaluation is challenging, and the correlations between DOI and POI are unknown. We aimed to assess DOI and worst pattern of invasion (WPOI) in early-stage OSCC, and evaluate the preoperative predictive ability of biopsy pattern of invasion (BPOI) for WPOI and DOI. This retrospective study included $n = 444$ OSCC patients with pT1-2N0M0. The prognostic value of DOI, WPOI, and BPOI and the predictive prognostic option for WPOI and DOI by BPOI were assessed. WPOI (1–3 vs 4–5), but not BPOI, predicted the lowest survival rate and highest DOI. To evaluate the difference between WPOI and BPOI, we conducted a POI type-matching analysis of patients with BPOI1–4 and WPOI1–5. Based on each WPOI type, the false-prediction rates (FPR) of BPOI types 1 ($n = 23$), 2 ($n = 89$), 3 ($n = 252$), and 4 ($n = 80$) were 52.17%, 52.81%, 36.90%, and 0%, respectively. BPOI4 perfectly predict WPOI 4–5. As the false-predicted BPOI 1–2 was almost WPOI2–3 (79.7%), regardless of the existed FPR, patients with BPOI1–2 have longer survival and lower DOI than those with BPOI 4. However, this phenomenon was not observed in BPOI3, because all false-predicted BPOI3 were WPOI4–5 with a high DOI. We provide an alternative predictive prognostic option for WPOI and DOI by evaluating BPOI during OSCC surgical planning, with the recommendation of conservative treatment in patients with BPOI 1–2.

Keywords Biopsy pattern of invasion; · Worst pattern of invasion; · Depth of invasion; · Oral squamous cell carcinoma; · Prognosis

Introduction

Oral squamous cell carcinoma (OSCC) is the commonest malignancy of the oral cavity, and surgical excision is its primary form of management. According to the National Comprehensive Cancer Network guidelines [1], curative tumor resection can be undertaken by using accepted criteria, such as margin status, for adequate excision and neck dissections, if needed [2–7]. The treatment plan for OSCC patients is usually based on the TNM classification. The 8th American Joint Committee on Cancer (AJCC) guidelines for oral cancer have incorporated the depth of invasion (DOI) into the T stage because it was a strong predictor for occult or late lymph node metastasis, tumor recurrence, and lower survival [8, 9]. The DOI is defined as the distance from the deepest level of invasion to the adjacent normal mucosal surface [10]. Importantly, the pattern of invasion (POI) has been regarded as a criterion for the tumor invasiveness in OSCC patients. The worst pattern of invasion (WPOI) refers to the worst manner of infiltration present at the tumor/host interface, and includes 5 types [11]: WPOI1–3 indicate a relatively low invasiveness, and WPOI of 4 and 5 indicated high invasiveness. There is evidence to increasingly demonstrate that, when compared to patients with WPOI1–3, patients with WPOI4 and 5 had higher rates of mortality, locoregional recurrence, and occult cervical metastasis [12–17], as well as decreased T cell infiltration [18]. Both DOI and WPOI reflect the invasiveness of

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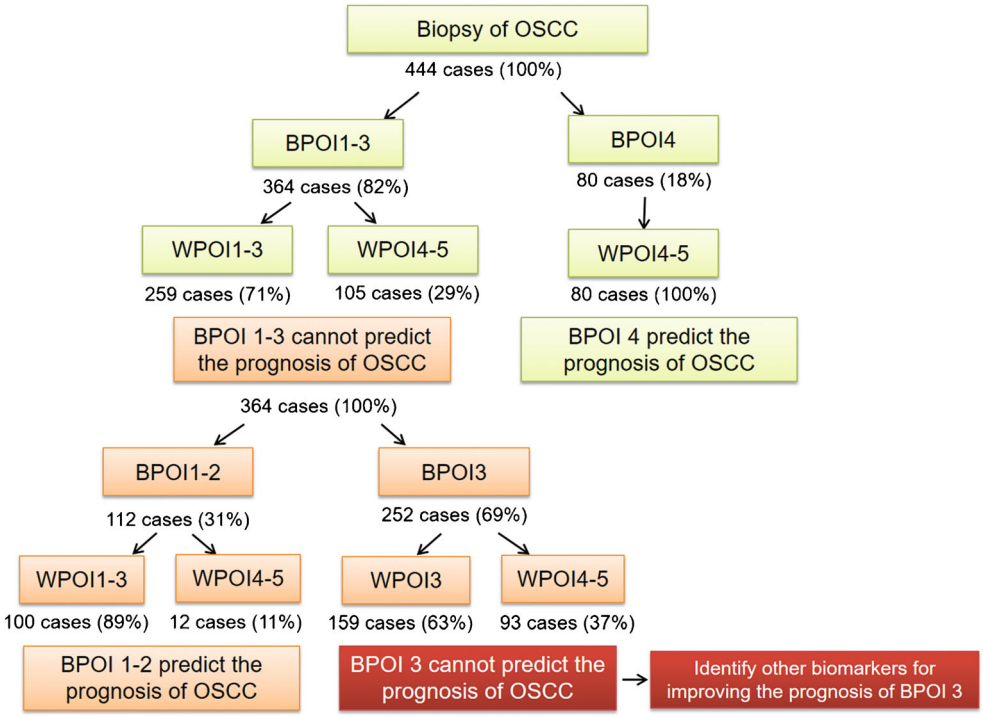

Fig. 1 Flow diagram illustrating the management of patients with OSCC stratified by the grade of BPOI and the prediction ability of WPOI by BPOI. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

the tumor cell. We hypothesized that the WPOI reflects the invasiveness of the tumor cell, whereas DOI may be a result of the POI. Therefore, correlations between the invasion pattern and invasion distance should be investigated, and could provide guidance for surgical planning.

At present, another obstacle is that the DOI and WPOI can only be evaluated postoperatively, which would not provide effective intraoperative guidance. A biopsy specimen can be obtained preoperatively and comprises some valuable prognostic indicators. Seki et al. found that the tumor budding score in the biopsy specimen is effective in predicting the prognosis in cN0 early-stage OSCC [19]. The prognostic value in the biopsy and surgical specimen is not always consistent, although the POI evaluation on a biopsy specimen—namely, the biopsy pattern of invasion (BPOI)—may provide timely information for intraoperative guidance, and may help surgeons preoperatively plan the extent of resection. However, because of the small area of the biopsy sample, the correlations between the DOI, WPOI, and BPOI are currently unclear, and the efficacy of prognosis prediction by BPOI needs to be investigated for improved surgical planning [15].

In this study, we aimed to analyze the correlation between DOI, WPOI, and BPOI in OSCC patients with pT1-2N0M0, and to evaluate the prognosis value of BPOI. Moreover, we intended to determine the ratio of false prediction of WPOI by BPOI, and to analyze whether the false prediction rate of BPOI affects the prognosis.

Materials and methods

Patients and pathological examination

This retrospective study enrolled 496 patients who were diagnosed with primary OSCC of pT1-2N0M0 at the Department of Pathology, Nanjing Stomatology Hospital, (Nanjing, China) from 2005 to 2014. We excluded 52 patients with missing data on the follow-up, and the final study cohort comprised 444 patients. Data on age at diagnosis, sex, site of lesion, TNM stage and tumor histologic grade, WPOI, BPOI, status of the margins, etc., were collected to analyze their prognostic value. The use of patient samples and data retrieval were approved by the Research Ethics Committee of Nanjing Stomatology Hospital, and informed consent was obtained from the patients.

All pathological assessments were performed by two experienced pathologists, neither of whom was aware of the clinical outcomes. The POI was examined at the host/tumor interface according to the POI types 1 through 5 as previously described by Bryne et al. [11, 20]. Briefly, POI Type 1 represents tumor invasion in a broad pushing manner with a smooth outline; POI Type 2 represents tumor invasion with broad pushing fingers or separate large tumor islands, with a stellate appearance; POI Type 3 represents invasive islands that comprise more than 15 tumor cells/island; POI Type 4 represents invasive tumor islands comprised of fewer than 15 tumor cells/island; and POI Type 5 represents tumor satellites of any size with 1 mm or more of intervening normal tissue

Table 1 Clinical pathologic characteristics of OSCC patients

Characteristics	*n*	%
Total	444	100.0
Gender		
Male	221	49.8
Female	223	50.2
Age		
<60	200	45.0
≥60	244	55.0
Smoking		
No	127	28.6
Yes	317	71.4
Depth of invasion		
< 5 mm	267	60.1
≥ 5 mm	177	39.9
Differentiation		
Well	239	53.8
Medium to poor	205	46.2
T stage		
T1	197	44.4
T2	247	55.6
Margin status		
Negative	357	80.4
Mild dysplasia	52	11.7
Moderate dysplasia	21	4.7
Severe dysplasia and positive margins	14	3.2
WPOI		
WPOI1-3	257	57.9
WPOI4-5	187	42.1
BPOI		
BPOI1-3	364	82.0
BPOI4	80	18.0

OSCC oral squamous cell carcinoma, *WPOI* worst pattern of invasion, *BPOI* biopsy pattern of invasion

(not fibrosis) at the tumor/host interface (includes cordlike and single cell invasion). The highest POI score identified was noted as the WPOI, regardless of how focal. The worst pattern of invasion observed in the biopsy sample was defined as the BPOI.

DOI is measured by first finding the "horizon" of the basement membrane of the adjacent squamous mucosa. A perpendicular "plumb line" is established from this horizon to the deepest point of tumor invasion, which represents DOI.

Biopsy specimens were obtained in the margins of a typical area that include normal appearing tissue and lesions of 5 mm or more that appear malignant. Specimens should include horizontal margins of 8 mm or more and vertical margins of 5 mm or more to observe the front of the invasion.

Study design

The OSCC patients were stratified by the grade of BPOI and the predictive ability of the WPOI by BPOI (Fig. 1). Firstly, we divided the 444 OSCC patients into two groups, BPOI1–3 and BPOI4, to predict WPOI4–5 and indicate an advanced prognosis and DOI in OSCC. However, BPOI 1-3 (n = 364) could not predict the prognosis of OSCC. Second, we divided the 364 OSCC patients into two groups, BPOI1-2 and BPOI3, to evaluate whether the BPOI could predict the prognosis and DOI.

Statistical analysis

Correlations between the histopathological risk model and relevant clinical and tumor variables were assessed by cross-tabulation and the chi-square test. For the statistical analyses, low and intermediate risk were grouped together and compared with high risk. Survival curves were constructed based on the Kaplan–Meier method and compared with the log-rank test. For multivariate survival analysis, the Cox proportional hazard model with a stepwise method that included all variables was employed. The level of significance was set at 5% (p<0.05). Statistical analyses were conducted in SPSS 17.0 and Prism 5.0.

Results

The relevant clinicopathologic variables are shown in Table 1. The median age at diagnosis was 59.6 years (range: 30–88 years), and the median duration of follow-up was 43.8 months (range 7–126 months). Tumors were graded as well differentiated (53.8%) or moderately and poorly differentiated (46.2%). T1 tumors were diagnosed in 197 patients (44.4%) and T2 in 247 (55.6%). With regard to the DOI, there were 267 (60.1%) patients with DOI <5 mm and 177 (39.9%) patients with DOI >5 mm. Margins with dysplasia, positive margins (invasive tumor at margins), or both were observed in 19.6% of patients (mild, 11.7%; moderate, 4.7%; severe dysplasia and positive margins, 3.2%). None of the patients underwent pre- or postoperative chemotherapy and/or radiotherapy before recurrence or metastasis. Biopsy specimens included in the study had margins with normal-appearing tissue and lesions ≥5 mm that appeared malignant on an observation of the pattern of invasion.

BPOI is not an independent indicator for OSCC prognosis

We first investigated the prognostic value of WPOI, BPOI and DOI in OSCC patients with pT1-2N0M0 (n = 444). Kaplan–

Table 2 Prognostic factors for overall survival

Factors	OS					
	HR	Univariate 95 % CI	Sig.	HR	Multivariate 95 % CI	Sig.
Sex Men vs. Women	0.671	0.399–1.128	0.132			
Age <60 vs. ≥60	1.326	0.788–2.234	0.288			
Smoke No vs. Yes	0.790	0.432–1.662	0.574			
Differentiation Well vs. Medium to poor	1.995	1.372–3.399	0.011	1.381	0.806–2.365	0.240
DOI <5 mmvs≥5 mm	2.787	1.661–4.676	0.0001	2.163	1.192–3.928	0.014
Margin Status Negative vs. positive	4.744	1.429–15.740	0.011	1.969	0.883–4.394	0.098
POI WPOI 1-3 vs. 4-5	3.425	2.011–5.833	< 0.0001	2.449	1.375–4.363	0.002
POI BPOI 1-3 vs. 4	1.782	0.962–3.303	0.066			

Meier survival analysis revealed that WPOI4–5 and DOI ≥5 mm were correlated with lower overall survival (OS), recurrence-free survival (RFS), and disease-free survival (DFS) (Fig. 2a–c and d–f). Cox regression analysis indicated that the WPOI and DOI were an independent prognostic factor for OS, RFS, and DFS of OSCC (Tables 2, 3, 4). We further analyzed the correlation between WPOI and DOI. The results indicated that patients with WPOI1–3 had low DOI value, and WPOI4–5 predicted high DOI value; this indicated that patients with WPOI4–5 should be considered for radical surgery when compared with patients who have a WPOI of 1–3 in the same background (Table 5).

However, on Kaplan–Meier survival analysis, BPOI (1–3 vs 4) was unrelated to OS, RFS, or DFS (p=0.066, p=0.117, and p=0.088, respectively; Fig. 2g–i), and it could not predict the prognosis of OSCC patients (Tables 2, 3, 4).

BPOI types 1–3 could not efficiently predict WPOI1–3

To uncover a potential explanation of the different prognostic values of WPOI and BPOI, we sought to evaluate the false prediction rate (FPR) of the BPOI for WPOI. We found that patients were preoperatively diagnosed as BPOI1, although

Table 3 Prognostic factors for recurrence-free survival

Factors	RFS					
	HR	Univariate 95 % CI	Sig.	HR	Multivariate 95 % CI	Sig.
Sex Men vs. Women	0.913	0.560–1.489	0.715			
Age <60 vs. ≥60	1.121	0.686–1.832	0.648			
Smoke No vs. Yes	1.138	0.607–1.871	0.657			
Differentiation Well vs. Medium to poor	1.802	1.085–2.991	0.023	0.900	0.541–1.495	0.683
DOI <5 mmvs≥5 mm	2.094	1.285–3.412	0.003	1.726	1.008–2.956	0.047
Margin Status Negative vs. positive	8.165	2.848–23.41	< 0.0001	2.834	1.460–5.502	0.002
POI WPOI 1-3 vs. 4-5	2.234	1.355–3.683	0.002	1.838	1.091–3.097	0.022
POI BPOI 1-3 vs. 4	0.609	0.328–1.133	0.117			

Table 4 Prognostic factors for disease-free survival

Factors	DFS					
	HR	Univariate 95 % CI	Sig.	HR	Multivariate 95 % CI	Sig.
Gender Men vs. Women	0.834	0.0.534–1.305	0.427			
Age <60 vs. ≥60	1.201	0.767–1.881	0.423			
Smoke No vs. Yes	1.125	0.659–1.855	0.758			
Differentiation Well vs. Medium to poor	1.567	0.998–2.459	0.0511	1.149	0.723–1.826	0.558
DOI <5 mmvs≥5 mm	2.155	1.380–3.367	0.0007	1.644	1.004–2.691	0.028
Margin Status Negative vs. positive	9.253	3.447–24.84	< 0.0001	2.652	1.442–4.877	0.002
POI WPOI 1-3 vs. 4-5	2.732	1.730–4.315	< 0.0001	2.153	1.327–3.493	0.002
POI BPOI 1-3 vs. 4	0.598	0.335–1.079	0.088			

these results were inaccurate. Furthermore, the BPOI1 could actually be WPOI 1, 2, and 3 when diagnosed by postoperative pathology (Fig. 3a–c); BPOI2 could actually be WPOI 2, 3, and 4 (Fig. 4a–c); BPOI3 could actually be WPOI3 and 4 (Fig. 5a–b). All of the BPOI4 were WPOI4, and this cohort had the highest predictive efficacy (Fig. 5c). The results of statistical analysis are shown in Table 6; of the 80 BPOI Type 4 cases, the matching WPOI were all types 4 and 5, whereas the FPR of the BPOI type for WPOI4–5 was 0%. Among the BPOI Type 3 patients (n = 252), the FPR for WPOI3 was 37.70% (n = 95), and all of the falsely predicted BPOI3 were WPOI4 and 5.

Among BPOI Type 2 patients (n = 89), the FPR for WPOI3 and 4–5 was 52.81% (n = 47). Of the 47 OSCC patients, 35 were WPOI3 and 12 patients were WPOI4–5. Among the BPOI1 cases (n = 23), five patients actually belonged to the WPOI3 type, and seven belonged to WPOI2; the FPR for WPOI1 was 52.17% (n = 12). These results demonstrated that the BPOI Type 4 could perfectly predict WPOI4–5, whereas BPOI types 1–3 could not efficiently predict the WPOI1–3, respectively.

Unexpectedly, patients with BPOI1–3 had a comparable DOI value than those with BPOI4, and a favorable DOI could not be predicted (Table 5).

Table 5 The correlation between WPOI, BPOI, and DOI

		DOI(mm)		Median	P value
		≤Median	>Median		
WPOI	1–2	50	10	4.2	0.001
	4–5	74	113		
	3	119	78	4.2	0.027
	4–5	75	112		
	13	155	102	3.9	0.001
	4–5	68	119		
BPOI	1–2	69	44	3.45	0.001
	4	28	52		
	3	131	121	4.3	0.444
	4	37	43		
	1-3	46	175	3.9	0.161
	4	34	189		

BPOI types 1–2 sufficiently predict the prognosis of OSCC patients

The inconsistency between the patient numbers of BPOI and WPOI at each type was not simply taken to mean that the BPOI had no potential prognostic value, because most of the falsely predicted BPOI1–2 (n = 59) were WPOI2–3 (n = 47, ratio: 79.7%), whose clinical outcomes were relatively better. However, 95 patients with BPOI Type 3 classification actually belonged to the WPOI4–5 type, whose clinical outcomes were relatively poor (Table 6). Thus, we next aimed to determine the DOI value and prognosis of BPOI types 1–2 and 3.

The results indicated that both patients with WPOI types 1–2 or 3 showed relatively longer survival times than whose with WPOI types 4–5 (Fig. 6). Interestingly, patients with BPOI types 1–2 grading had longer OS, DFS, and RFS than patients with BPOI Type 4. Interestingly, patients with BPOI1–2 had low DOI value, and the BPOI4 predicted a high DOI value, thereby indicating that patients with BPOI1–2 should be considered for conservative surgery as compared to patient with BPOI4 in the same background (Table 5).

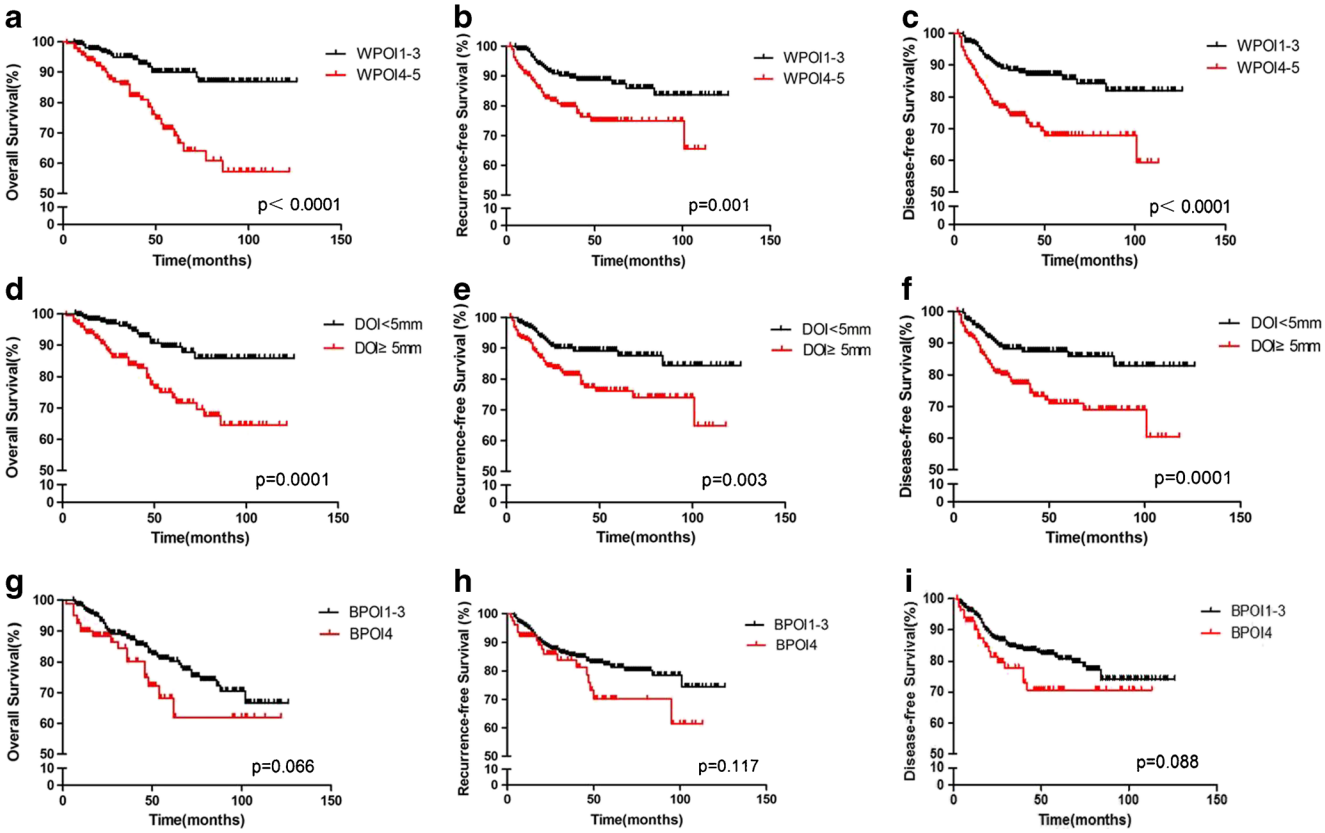

Fig. 2 Kaplan–Meier plots of **a** overall survival of OSCC patients with WPOI1–3 vs WPOI4–5, **b** recurrence-free survival of OSCC patients with WPOI1–3 vs WPOI4–5, **c** disease-free survival of OSCC patients with WPOI1–3 vs WPOI4—5; **d** overall survival of OSCC patients with DOI <5 mm vs DOI ≥5 mm, **e** recurrence-free survival of OSCC patients with DOI <5 mm vs DOI ≥5 mm, **f** disease-free survival of OSCC patients with DOI <5 mm vs DOI ≥5 mm, **g** overall survival of OSCC patients with BPOI1–3 vs BPOI4, **h** recurrence-free survival of OSCC patients with BPOI1–3 vs BPOI4, **i** disease-free survival of OSCC patients with BPOI1–3 vs BPOI4. Patients with WPOI1–3 have better prognosis than those with WPOI4–5 and showed longer survival time, patients with DOI <5 mm have better prognosis than those with DOI ≥5 mm and showed longer survival time. However, BPOI (1–3 vs. 4) was not significantly associated with OSCC prognosis. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

However, patients with BPOI types 3 and 4 had comparable DOI value and survival time, including the OS, DFS, and RFS (Fig. 7), which could be explained by the fact that 95 patients with BPOI type 3 cases actually belonged to the WPOI4–5 group, with a high DOI. These findings implied that the false-predicted BPOI1–2 showed an influence on patient survival and could effectively predict the DOI preoperatively, although greater efforts should be devoted to improving the prognostic value of BPOI3 through the use of other biomarkers (Table 5).

Thus, the 444 OSCC patients into two groups, BPOI1–3 and BPOI4, showed that BPOI4 (n = 80) could perfectly predict WPOI4–5 and indicate an advanced prognosis and DOI in OSCC. However, BPOI1–3 (n = 364) could not predict the prognosis of OSCC. We divided the 364 OSCC patients into two groups: BPOI1–2 and BPOI3, and found that BPOI1–2 could predict the prognosis and DOI, whereas BPOI3 could not predict the prognosis.

Table 6 The patient numbers in each type of WPOI and BPOI

BPOI type	WPOI type (n/%)				
	1	2	3	≥4	Total
1	11 (47.83)	7 (30.43)	5 (21.74)	0 (0.00)	23
2	0 (0.00)	42 (47.19)	35 (39.33)	12 (13.48)	89
3	0 (0.00)	0 (0.00)	157 (62.30)	95 (37.70)	252
4	0 (0.00)	0 (0.00)	0 (0.00)	80 (100.00)	80
Total	11	49	197	187	444

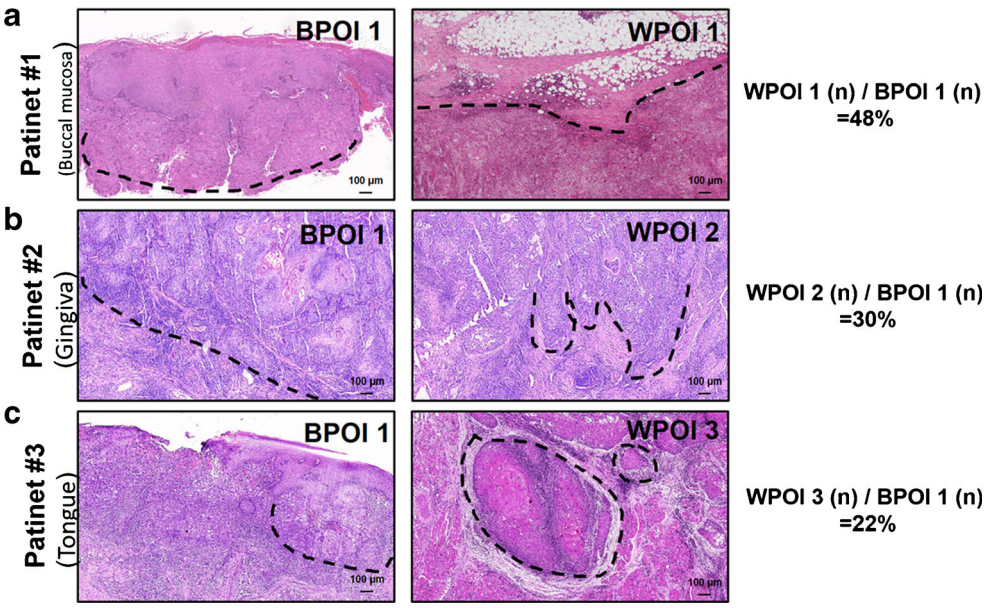

Fig. 3 The grade of WPOI of OSCC patients with BPOI1. **a** The BPOI1 of Patient 1 showed concordance with the corresponding resection specimen, which also showed a WPOI1; **b** the BPOI1 of Patient 2 showed discordance and WPOI2; **c** the BPOI1 of Patient 2 showed discordance and WPOII. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

Discussion

The DOI is a significant predictor of OSCC. Several studies have shown a relationship between DOI and lymph node metastasis in early-stage OSCC [21–23]. Faisal M and Chandler K et al. found that the DOI is associated with significantly increased risk of local recurrence [9, 24]. Therefore, the DOI was incorporated as a modifier to the T category for the OSCC in the AJCC8 in 2017 [25]. Thus, the DOI can possibly guide the treatment plan, including the extent of resection and neck dissection. However, DOI can be postoperatively measured on the pathological analysis. The results of preoperative B ultrasound and MRI are inaccurate because of the small tumor outgrowths at the front of the tumor, which might be attributed

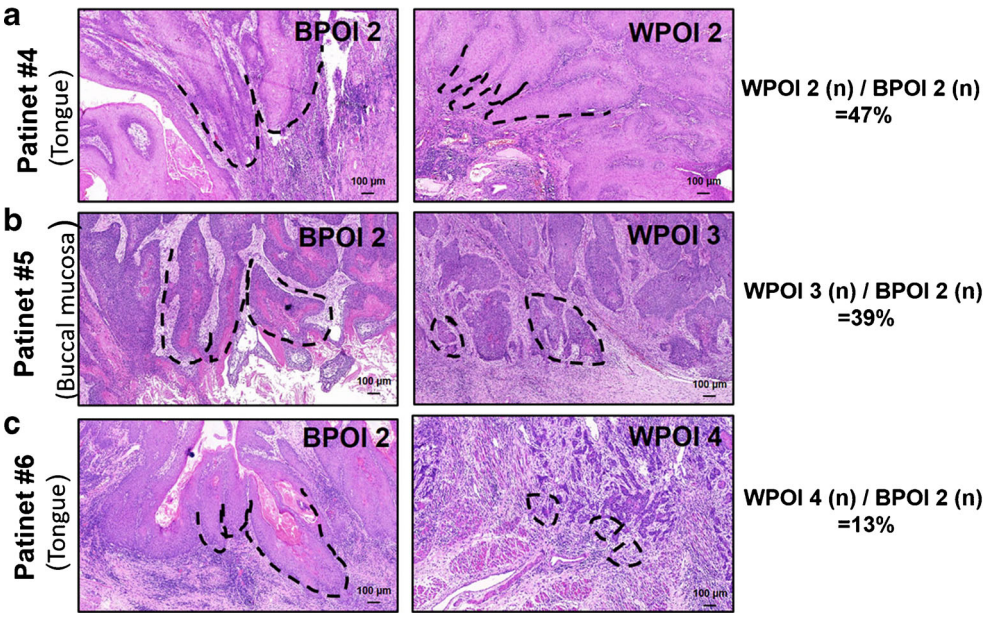

Fig. 4 The grade of WPOI of OSCC patients with BPOI2. **a** The BPOI2 of Patient 4 showed concordance with the corresponding resection specimen and a WPOI2; **b** The BPOI2 of Patient 5 showed discordance and a WPOI3. **c** The BPOI2 of Patient 6 showed discordance and a WPOI4. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

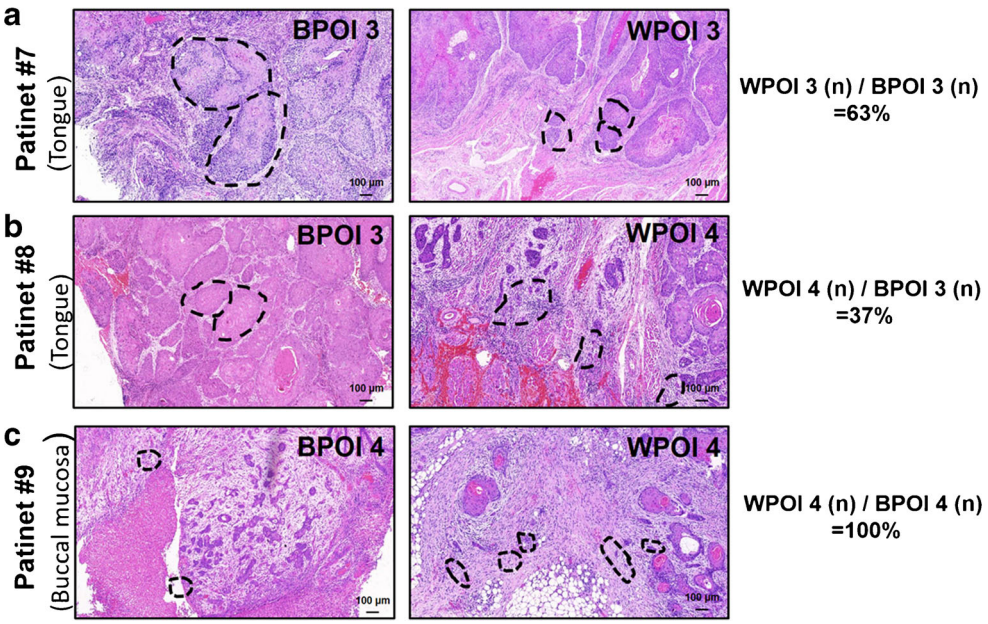

Fig. 5 The grade of WPOI of OSCC patients with BPOI3 and BPOI4. **a** The BPOI3 of Patient 7 showed concordance with the corresponding resection specimen and a WPOI3; **b** the BPOI3 of Patient 8 showed discordance and a WPOI4; **c** The BPOI4 of Patient 9 showed concordance with the corresponding resection specimen and a WPOI4. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

to an advanced pattern of invasion. However, the WPOI can be observed in the invasive margin, regardless of focal growth [11], and is considered to be another valuable predictor of OSCC prognosis as it can predict the local recurrence and occult neck metastasis [14–17]. In our recent research, we found that the invasiveness of both tumor and stroma cells in WPOI4–5 was stronger than that in WPOI1–3 (data not shown). In recent years, some studies have suggested that OSCC patients with WPOI4–5 need a wider resection to ensure a clear margin [26], as well as the need for neck dissection for patients with clinically node-negative disease [27]. Therefore, the DOI seems to be the result of the WPOI. In this study, we found that patients with WPOI1–3 had low DOI value, and WPOI4–5 predicted high DOI value, indicating that patients with WPOI4–5 should be considered for radical surgery when compared with patients with WPOI1–3 in the same background. However, the existing studies have focused mainly on the WPOI of the surgical specimen, which could only be evaluated postoperatively, thereby reducing the preoperative value of POI and DOI.

Biopsy specimens can be obtained preoperatively and comprise some valuable prognostic factors. In colorectal cancer, Parks suggested that the assessment of the TME is comparable in biopsy and surgically resected specimens from patients, and biopsy-based assessment could facilitate preoperative stratification or commencement of therapy that targets the TME [28]. In endometrial carcinoma, a high level of concordance was achieved between biopsy and hysterectomy specimens for mismatch repair protein (MMR)-loss, microsatellite instability (MSI)-high, P53-wild and abnormal types, superior to that of grade and histologic subtype, providing earlier and more reliable prognostic information for the clinical management plan [29]. In cN0 early-stage OSCC, Seki et al found the tumor budding score in the biopsy sample can effectively predict the prognosis [19]. However, the tumor budding was difficult to assess consistently in different slides of a sample for the small nest (less than 5 cells) and the closed cut-off value (≥5 buddings refers to high budding score, 3–4 intermediate budding score, ≥5 high budding score) [19]. While in our article, we found that the pattern of invasion could also been evaluated in the biopsy sample, which we call BPOI. Differ from tumor budding, the evaluation of BPOI was reproducible.

However, the correlation between the invasion pattern of the biopsy sample and WPOI and DOI in the surgical specimen has not yet been studied [15]. In the present study, we

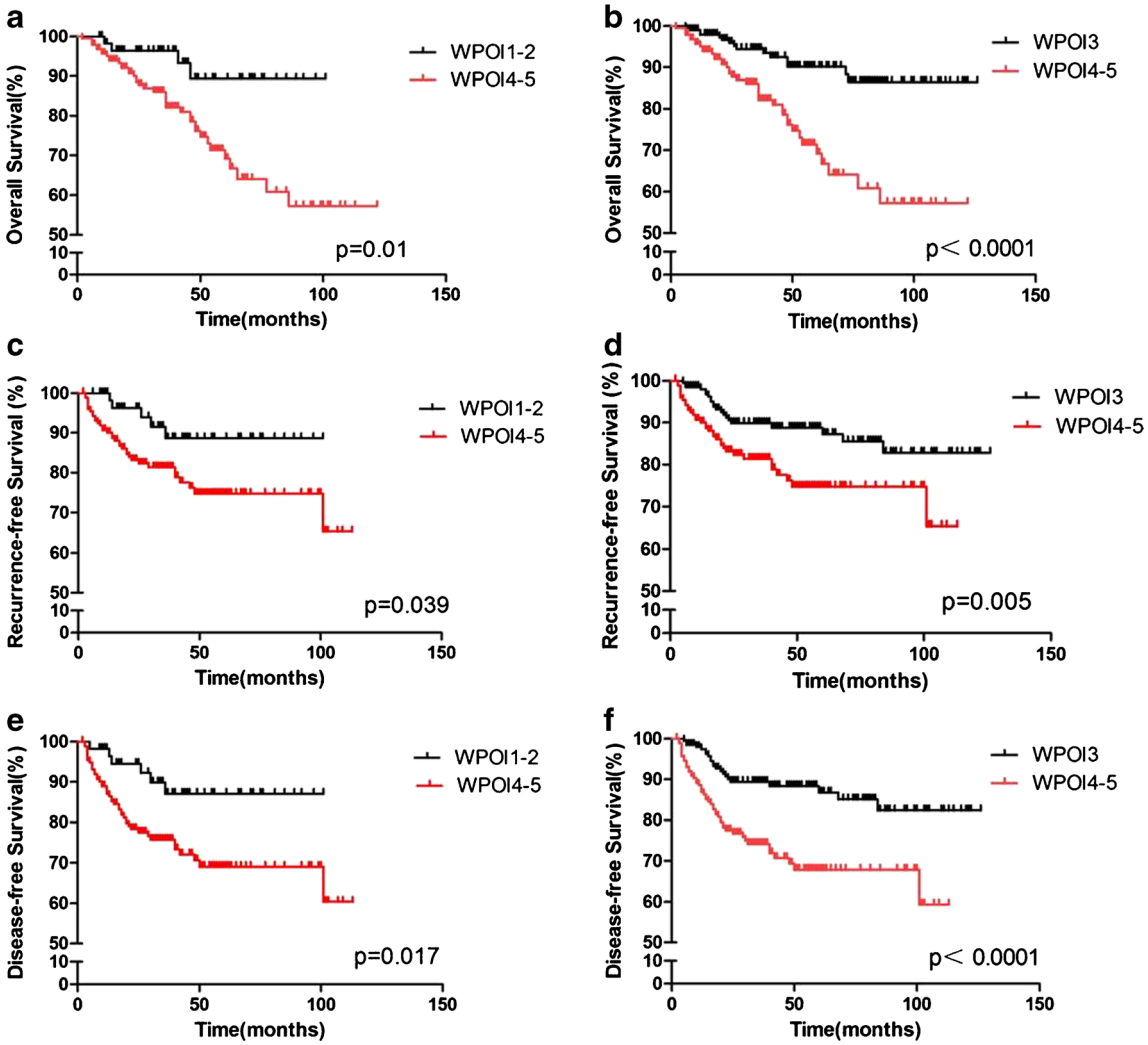

Fig. 6 Kaplan–Meier plots of **a** overall survival of OSCC patients with WPOI1–2 vs WPOI4-5; **b** overall survival of OSCC patients with WPOI3 vs WPOI4–5; **c** recurrence-free survival of OSCC patients with WPOI1–2 vs WPOI4–5; **d** recurrence-free survival of OSCC patients with WPOI3 vs WPOI4–5; **e** disease-free survival of OSCC patients with WPOI1–2 vs WPOI4–5; **f** disease-free survival of OSCC patients with WPOI3 vs WPOI4–5. Patients with WPOI1–2 and WPOI3 have better prognosis than those with WPOI4–5 and showed longer survival time. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

analyzed the relationship between WPOI and DOI and found that, in early-stage OSCC, the DOI can vary according to the POI. However, although the BPOI can be obtained from the biopsy specimen, 93 patients with BPOI type 3 grading were actually WPOI4–5, whose clinical outcomes were relatively poor and were associated with a high DOI. Thus, in OSCC patients, the BPOI3 can neither predict the prognosis nor the DOI. However, in the remaining patients, BPOI4 could effectively predict WPOI4–5, and BPOI1–2 is associated with better prognosis as well as a low DOI than BPOI4, indicating that patients with BPOI1–2 should be considered for conservative surgery in the same background.

To our knowledge, this study is the first to investigate the clinical value of BPOI via the prediction of the WPOI and DOI. The results demonstrated that patients with BPOI1 and 2 have low DOI value and longer OS and DFS than those with

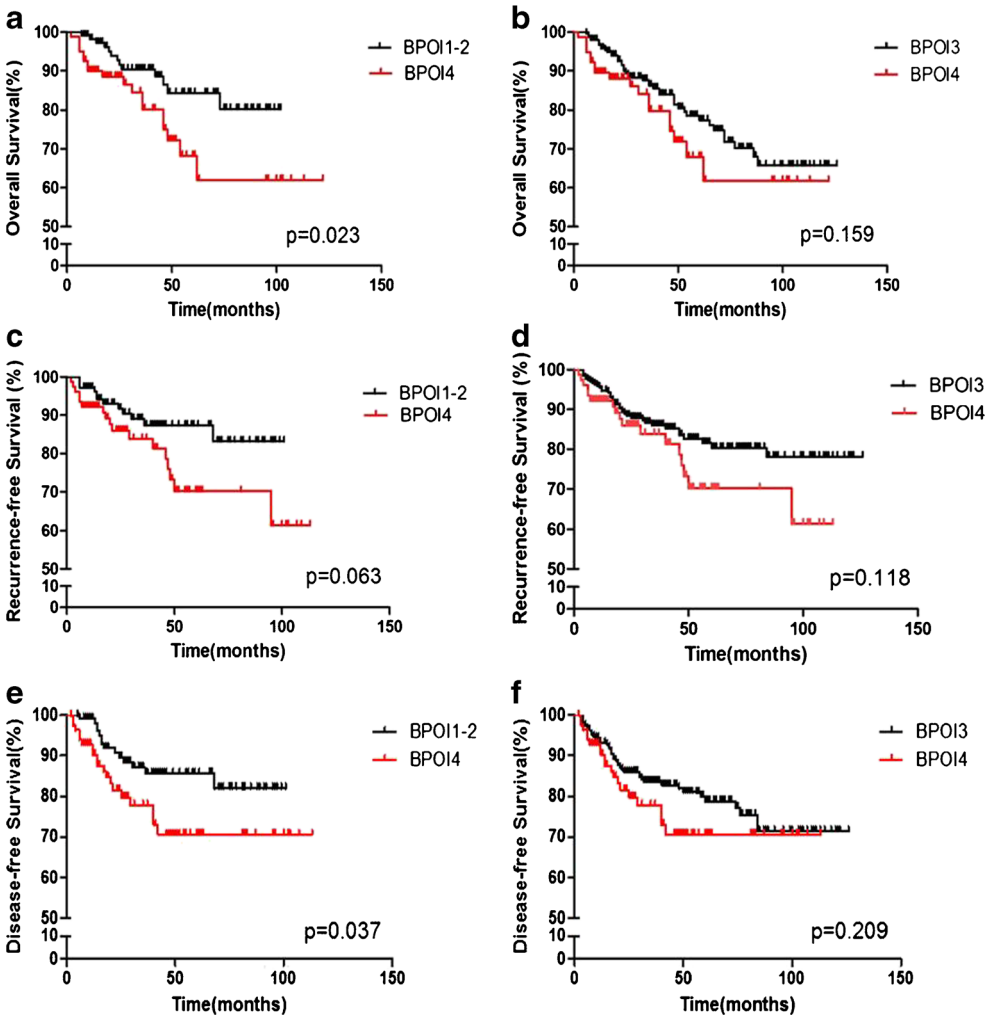

Fig. 7 Kaplan–Meier plots of **a** overall survival of OSCC patients with BPOI1–2 vs BPOI4; **b** overall survival of OSCC patients with BPOI3 vs BPOI4; **c** recurrence-free survival of OSCC patients with BPOI1–2 vs BPOI4; **d** recurrence-free survival of OSCC patients with BPOI3 vs BPOI4; **e** disease-free survival of OSCC patients with BPOI1–2 vs BPOI4; **f** disease-free survival of OSCC patients with BPOI3 vs BPOI4. Patients with BPOI1–2 have better prognosis than those with BPOI4; however, patients with BPOI 3 and 4 have comparable survival time, regardless of overall survival time, disease-free survival, and recurrence-free survival. OSCC, oral squamous cell carcinoma; BPOI, biopsy pattern of invasion; WPOI, worst pattern of invasion

BPOI4, regardless of the FPR. In combination with imaging investigations such as MRI and B ultrasound, BPOI1–2 and 4 can guide clinicians in developing the treatment plan of patients with OSCC. Further research should emphasize mechanisms to improve the predictive efficacy of BPOI3 in association with other diagnostic markers.

Authors' contributions Yanhong Ni and Qingang Hu contributed to the conception of the study, Yumei Pu and Liang Ding designed the study and analyzed the data and were major contributors in writing the manuscript, Yujia Wang and Yuxin Wang analyzed and interpreted the patients' data. Sheng Chen and Xiaofeng Huang performed the histological examination of the OSCC, Zhifeng He helped perform the analysis with constructive discussions, All authors read and approved the final manuscript.

Funding This study was supported by National Natural Science Foundation of China (No. 81902754, 81772880). China Postdoctoral Science Foundation (No. 2019 M651789). Fundamental Research Funds for the Central Universities (No. 021014380117). Natural Science Foundation of Jiangsu Province (No. BK20190304). General projects in the development of medical technology in Nanjing (No. YKK16158). Nanjing Medical Science and technique Development Foundation (No.QRX17174).

Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable

Consent to participate (include appropriate statements) Not applicable

Consent for publication (include appropriate statements) Not applicable

Code availability (software application or custom code) Not applicable

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