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Predictive Value of Emerging Histopathological Parameters for Lymph Node Metastasis in Oral Squamous Cell Carcinoma: A Retrospective Cohort Study

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Abstract

Background

Cervical lymph node metastasis is a major prognostic factor in oral squamous cell carcinoma (OSCC), yet accurate prediction remains challenging. Histopathological features at the invasive tumor front, including tumor budding (TB), worst pattern of invasion (WPOI), and lymphocytic host response (LHR), have emerged as potential indicators of tumor aggressiveness but are not routinely incorporated into reporting protocols. This study evaluated, using digitized histopathological slides, the predictive value of these parameters alongside established pathological factors for lymph node metastasis and survival outcomes in OSCC.

Material and Methods

A retrospective cohort study was conducted on 89 OSCC patients treated by surgical resection and neck dissection from 2016 till 2025 at Alexandria university. Histopathological slides were retrieved, scanned and reviewed to assess TB, WPOI, LHR, depth of invasion (DOI), perineural invasion (PNI), lymphovascular invasion (LVI) and extranodal extension (ENE). Univariate and multivariate logistic regression analysis were conducted to determine the predictive factors for lymph node metastasis (LNM). Receiver operating characteristic (ROC) analysis was performed to identify the optimal DOI cut-off value. Overall survival (OS) and locoregional recurrence free survival (LRRFS) were evaluated using Kaplan Meier analysis and Cox proportional hazards regression.

Results

Lymph node metastasis was significantly associated with increased DOI, high TB, aggressive patterns of invasion, limited LHR. Tumor budding was the only independent predictor for LNM in the multivariate analysis (aOR = 7.63, p value < 0.001). ROC analysis identified 7 mm as the optimal DOI cut-off value for predicting nodal metastasis in our cohort. Advanced AJCC7th pT stage was associated significantly with reduced OS and LRRFS, while LVI was associated with reduced LRRFS.

Conclusions

Tumor budding is a robust and independent predictor of lymph node metastasis in OSCC and its incorporation into routine histopathological reporting may enhance risk stratification. DOI remains clinically valuable for guiding neck managements decisions, although the implications of DOI-based upstaging should be interpreted cautiously. While WPOI-4 and limited LHR warrant greater consideration as potential markers for tumor aggressiveness.

Background

Oral cancer is the 16th most common cancer worldwide (1). According to GLOBOCAN estimates, approximately 387,485 new cases and 188,230 deaths from lip and oral cancers were reported in 2022

(2). In Egypt, lip and oral cavity cancer ranks 18th in both incidence and mortality, with 1,578 new cases and 659 deaths reported in 2022; the crude rates are projected to double by 2030 (3). Oral squamous cell carcinoma (OSCC) constitutes more than 90% of all oral malignancies (4).

Oral squamous cell carcinoma commonly metastasizes via lymphatic drainage to the ipsilateral lymph nodes of the neck; contralateral and bilateral neck involvement can also occur. The overall survival is reduced by 50% when cervical lymph nodes are involved, with a higher incidence of distant metastasis involving mostly the lung, bone and liver, thus making metastatic cervical nodal involvement the most significant prognostic factor in OSCC (5, 6). Consequently, proper management of the cervical lymph nodes is as critical as treatment of the primary site (7).

The treatment plan and estimated prognosis of OSCC patients mainly depend on the TNM staging system. The National Comprehensive Cancer Network (NCCN) and the Union for International Cancer Control (UICC) guidelines recommend a single treatment modality for early-stage (I-II) patients, whereas advanced-stage patients (III-IV) often require more aggressive multimodal therapy (8, 9). Heterogeneity in the survival within the same tumor stage suggests that anatomical staging alone does not fully capture the biological behavior of the disease (10).

Accurate assessment of cervical lymph node status remains a major challenge in OSCC management. Despite advances in clinical examination and imaging techniques, occult metastasis is identified in approximately 20–50% of the node-negative patients, with many subsequently developing cervical metastasis within 2 years of follow-up. In contrast, lymph nodes classified as clinically positive do not always harbor metastatic disease upon pathological review, as reactive or inflammatory swelling can frequently simulate malignant features (6, 11, 12). Consequently, the controversy between elective neck dissection and "watch and wait" approach remains unresolved for the management of early-stage disease (7). Unfortunately, even these early stages of the tumor may act aggressively, exhibiting a high tendency to invade and metastasize, resulting in a poor prognosis (13). On the other hand, the routine application of elective neck dissection (END) to all patients may results in overtreatment and increase complications-related morbidity (6).

Therefore, patients with a high risk of metastasis should be identified, and at the same time, unnecessary surgery should be avoided in patients who are unlikely to be harboring a metastasis (11).

Recognizing the importance of histopathological tumor features, the American Joint Committee on Cancer (AJCC), in its 8th edition, incorporated depth of invasion (DOI) into the T category and extranodal extension (ENE) into the N category as critical parameters in staging, to improve prognostic accuracy and predictability. Additionally, NCCN guidelines advocate the role of adverse pathological features such as perineural invasion (PNI) and lymphovascular invasion (LVI) as indicators for postoperative radiotherapy (PORT) (8, 14). Despite these adjustments, the 8th edition remains limited in its ability to identify patient with a poor prognosis (15–17). Yet, recognizing the multidimensional biological behavior underlying nodal metastasis is essential for accurate treatment planning and postoperative care(18).

The invasive tumor front has gained attention as the residence for the most aggressive tumor cells responsible for invasion, spreading, and metastasis (19–23). Histopathological parameters such as tumor budding (TB), worst pattern of invasion (WPOI), and lymphocytic host response (LHR) reflect tumor aggressiveness and host interaction. Tumor budding (TB), defined as an isolated cell or a small cluster of cells up to four cells at the invasive front, has emerged as a powerful prognostic predictor for lymph node metastasis (LNM) and prognosis in colorectal, lung, pancreatic, and esophageal cancer (24–27). WPOI is another form of architectural aggressiveness of the tumor at the invasive front, while lymphocytic host response reflects the interaction between tumor cells and host immune system (28).

Consequently, clinical decision-making should be evaluated based on a combination of these biological features rather than relying on a single isolated parameter (18).

Although these histopathological parameters in OSCC have shown promising prognostic value, their integration into routine pathology reporting remains inconsistent. Some are relegated to non-core or optional elements, while others are excluded altogether, underscoring a critical gap between emerging evidence and standardized diagnostic practice(29).

The aim of this study is to evaluate the predictive and prognostic value of the histopathological parameters at the invasive tumor front, including tumor budding, worst pattern of invasion, and lymphocytic host response, for lymph node metastasis and survival outcomes, alongside established factors such as depth of invasion (DOI), extranodal extension (ENE), perineural invasion(PNI), and lymphovascular invasion(LVI) in a cohort of Egyptian population to support the integration of these parameters into standardized reporting systems, thereby refining decision making and improving prognostic assessment in OSCC.

Materials and Methods

After gaining the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University (IRB No. 00010556- IORG 0008839), Ethical NO. (1062-04/2025). A retrospective cohort study was conducted using archival material from Oral Pathology Department, Faculty of Dentistry, Alexandria University, and Pathology Department, Faculty of Medicine, Alexandria University. Cases of oral squamous cell carcinoma (OSCC) diagnosed between 2016 and 2025 were identified. Eligible patients were those who underwent surgical resection of the primary lesion along with neck dissection of the lymph nodes. Patients were excluded if they had received chemotherapy or radiotherapy prior to surgery(n = 4), presented with a recurrent lesion (n = 10), or had a history of other malignancies within the preceding five years (n = 3). Cases with incomplete medical records or incomplete sets of pathological slides for both the resected tumor and dissected nodes were also excluded (n = 77).183 resected OSCC with lymph nodes cases were identified in the records, 89 patients met the inclusion criteria and were included in the study.

Diagnostic reports and documents indicating lymph node status were withheld. All cases were anonymized, coded, and randomly shuffled before evaluation.

A subset of 30 randomly selected cases was independently assessed by two observers to evaluate interobserver reproducibility. The entire study cohort was evaluated by one observer and confirmed by the second observer. In cases of disagreement, both observers re-examined the slides and reached a consensus through discussion. Following the completion of the histopathological evaluation, decoding of the study data were applied and any discrepancies between the study assessments and the original pathology reports in the routinely reported parameters were re-evaluated and resolved through joint review and discussion.

Histopathological Parameters Evaluation

Hematoxylin and eosin (H&E) slides of the resected specimens and dissected lymph nodes were retrieved and scanned at 40x magnification setting using Motic Easy Pro whole-slide scanner at Borg El Arab University Hospital, Pediatric Oncology Center. Whole-slide images (WSI) were reviewed using Motic DSAssistant software to assess different histopathological parameters blinded to lymph node status.

As a supplementary investigation, immunohistochemical stating for pan-cytokeratin was performed to further characterize specific histopathological features.

Tumor size at the greatest dimension was recorded from the previous diagnostic reports in the gross examination.

For each case all the available slides for the primary resected tumor were reviewed and the slide with the deepest DOI was selected. DOI was measured by drawing a vertical line to the deepest point of invasion after reconstructing the basement membrane of the normal mucosa by a horizontal imaginary line and rounded to the closest millimeter (14, 30) (Fig. 1).

Figure (1) Whole slide images for OSCC. **(a)** An ulcer in the buccal mucosa, a straight line was drawn from the normal mucosa on both sides, and a perpendicular line was dropped to the deepest point of invasion. **(b-c)** OSCC related to the lateral border of the tongue, a convex arcuate line was drawn instead of straight horizontal line to reconstruct the natural contour of the tongue. **(c)** Gross examination (left picture) showing the convex natural contour of the tongue, H&E slide (middle picture), and pan cytokeratin immunohistochemical staining (right picture).

Tumor budding was assessed according to the International Tumor Budding Consensus Conference (ITBCC) 2016 (31) by drawing 10 circles each with a fixed area (0.785 mm^2) at the invasive front on WSI. The circle with the highest visual (hotspot) tumor budding was chosen to count the tumor buds (single tumor cell or a cluster up to 4 tumor cells (Fig. 2c). Buds counts 0–4 buds were categorized as low tumor budding (BD1), 5–9 buds categorized as intermediate tumor budding (BD2), and > 10 buds categorized as high tumor budding (BD3) (Fig .2, 3).

Figure (2) A case with an ulcer at the left lateral border of the tongue. (a) 10 circles (0.785 mm^2 each) were drawn at the invasive front of the tumor. **(b)** High tumor budding BD3; > 10 buds in the hot spot chosen circle (*), limited LHR and WPOI-4. **(c)** High magnification shows tumor buds as a single cell up to 4 cells (black arrows).

Figure (3) Pan-cytokeratin immunohistochemical staining demonstrated high tumor budding (BD3) at the invasive front of the tumor.

Worst Pattern of Invasion (WPOI) was evaluated according to the classification proposed by Brandwein Gensler et al. (28). **WPOI-1** describes tumor that invade with a broad, pushing border. **WPOI-2** refers to invasion in a finger-like pattern (Fig. 4a). **WPOI-3** involves invasive tumor islands containing more than 15 cells per cluster. **WPOI 4** is defined as smaller invasive islands of fewer than 15 cells convincingly separated from the main tumor (Fig. 2b, 4b). **WPOI 5** represents tumor islands of any size that are located at least 1 mm away from the primary tumor (Fig. 4c), or when a dispersion distance of 1 mm is observed between distally separated islands. WPOI-5 can also take the form of dispersed perineural or lymphovascualar invasion (32, 33) (Fig. 4d).

Lymphoid infiltrate at the tumor–host interface is categorized into three patterns. **Pattern 1 (strong)** is defined by a continuous, dense rim of lymphoid tissue along the tumor interface. **Pattern 2 (intermediate)** is assigned when dense lymphoid patches are present but distributed discontinuously; the presence of any patch qualifies as Pattern 2. **Pattern 3 (limited)** reflects either a minimal response without patch formation or a complete absence of lymphoid infiltration (33) (Fig. 2b, 4c).

Figure (4) (**a)** WPOI-2; finger like invasion pattern. **(b)** WPOI-4; convincingly separate invasive island less than 15 cell (black arrows). **(c)** A case with skip metastasis to level IV shows WPOI-5, limited LHR and DOI 15 mm. **(d)** WPOI-5 represented as perineural invasion away > 1 mm from the main tumor.

Perineural invasion was defined as either the tumor wraps around at least 33% of the nerve circumference or tumor cells detected within any of the 3 layers of the nerve sheath (34)(Fig. 4d, 5). Lymphovascular invasion was assessed when a cohesive tumor embolus with a smooth surface, surrounded by a clear space, was detected within an endothelial lined vessel or was attached to its wall (35, 36) (Fig. 5f).

Figure (5) Perineural and lymphovascular invasion in OSCC. (a-b) H&E and pan cytokeratin immunohistochemical staining show total encirclement of the nerve by malignant epithelial cells. **(c-d)** Encirclement of 33% or more the nerve diameter. **(e)** Intraneural PNI, malignant epithelial cells (*) invading large nerve epineurium layer. **(f)** Embolus of malignant cells within an endothelial lined vessel.

Lymph node status assessment

After complete evaluation of the histopathological parameters, the lymph node status was uncoded. Nodal histopathological status and the level of metastasis were collected retrospectively from the

original pathology reports. The patients were divided into pN0 (all lymph nodes are negative for metastatic deposits) and pN+ (at least one lymph node shows metastatic deposit at any level). Slides of the lymph node with metastatic deposits were scanned and evaluated for ENE.

Following the International Consensus Recommendations of Diagnostic Criteria and Terminologies for Extranodal Extension in Head and Neck Squamous Cell Carcinoma (37), ENE is considered positive (ENE+) in cases when (Fig. 6): 1) Complete transgression of the primary LN capsule with extension into the perinodal soft tissues. 2) Infiltration into reduplicated LN capsule. 3) Infiltrative tumour nests surrounded by adipocytes on multiple sides in the LN hilum. 4) Matted LNs with histological evidence of tumour crossing from one LN into another.

Tumor confined within the LN capsule, thickened LN capsule without intracapsular viable tumour cells, and Lymphovascular emboli or Perineural involvement are considered as negative ENE (ENE-) (Fig. 7).

Follow up

The patients follow up data were collected from the Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Alexandria University. Among the 89 cases, follow up data was available for only 46 case (51.69%).

Overall survival (OS) was calculated from the date of surgery to the date of death or the date of the last follow up visit. Locoregional recurrence free survival (LRRFS) was calculated form the time of surgery to the first reported local or regional recurrence.

Figure (6) Positive ENE metastatic lymph nodes. (a) Minor ENE, metastatic deposits interact with perinodal fat tissue. **(b)** Minor ENE, metastatic deposit presents within the duplicated capsule. **(c)** Major ENE > 2 mm. **(d)** Soft tissue deposit considered as major ENE.

Figure (7) Negative ENE metastatic lymph nodes. (a) Metastatic deposits confined within the lymph node. **(b)** Lymphovascular embolus of malignant epithelium cells within a vessel in the lymph node helium considered as negative ENE.

Statistical analysis

Normality of the continuous data was tested using Kolmogrov-Smirnov and Shapiro Wilk tests. Continuous data were described using mean, standard deviation (SD), median, interquartile range (IQR), minimum, maximum, while qualitative data were reported by frequency and percentage. Associations between clinicopathological features and lymph nodes metastasis were assessed by independent t, Mann-Whitney U, Chi-squared tests, or Chi-squared test with Fisher's Exact adjustment. Univariate and multivariate binary logistic regression models were performed to identify the predictors for LNM including (AJCC 7th pT stage, AJCC 8th pT stage, DOI, TB, PNI, LVI, LHR, and WPOI). Only parameters that were statistically significant were included in the multivariate analysis. Receiver operating

characteristic (ROC) curve was created to determine the DOI cut-off value for predicting lymph node metastasis. Sensitivity, specificity, Youden index J, and Area Under ROC Curve (AUC) were calculated. Univariate Cox proportional hazards regression models were used to assess the histopathological features (AJCC 7th pT stage, AJCC 8th pT stage, DOI, TB, PNI, LVI, ENE, WPOI, and LHR) associated with LRRFS and OS. Kaplan-Meier survival curves were generated to demonstrate time to recurrence and death according to the same histopathological parameters. Interobserver agreement was analyzed using Cohen's kappa statistic for categorical variables, weighted kappa for ordinal variables and intraclass correlation coefficient (ICC) for continuous variables.

Test statistics, odds ratios (OR), adjusted odds ratios (aOR), 95% confidence Intervals (CI), hazard ratio (HR), and adjusted hazard ratio (aHR) were reported. p value < 0.05 was considered statistically significant. Data were analyzed using IBM SPSS version 23.0 for Windows (SPSS Inc., Chicago, USA) and MedCalc Statistical Software version 19.0.5 (MedCalc Software bvba, Ostend, Belgium).

Results

Descriptive statistics

A total of 89 patients were included in this retrospective cohort study, with a mean age 56 ± 12.63 years. There were 45 females (50.56%) and 44 males (49.44%). Tongue was the most common site in our cohort 46.7% (41 cases) followed by the buccal mucosa 21 cases (23.6%). Forty-nine patients (55.1%) were pathologically node-negative (pN0), while 40 (44.9%) had metastatic lymph nodes (pN+). Bilateral neck dissection was performed for 4 patients (4.4%).

The median tumor size at the greatest dimension was 2.5 cm. According to the AJCC 7th edition, 77 cases (86.5%) were classified as pT1-T2 and 12 cases (13.48%) as pT3-T4. Following incorporation of DOI according to the AJCC 8th edition, 21 cases (23.6%) were upstaged to a pT3-pT4. The median DOI was 8 mm.

Regarding tumor budding; 45 cases (50.56%) were classified as BD1, 14 (15.7%) as BD2, and 30 (33.7%) as BD3. WPOI-4 was the most frequently observed invasion pattern, identified in 43 cases (34.2%), followed by WPOI 1–3 in 31 cases (34.83%) and WPOI-5 was identified in 24 cases (26.97%). By assessing LHR; 28 cases (31.5%) demonstrated a strong continuous band pattern, 37 (41.6%) showed an intermediate patchy pattern, and 24 (26, 97%) exhibited limited LHR. PNI and LVI were identified in 21 (23.6%) and 18 (20.2%) cases, respectively.

Among the 40 node-positive patients, the most frequently involved levels were level I and II observed in 52.5% and 50%, respectively. Level IV was involved in 5 cases (12.5%) while level V was involved in only 3 cases (7.5%). ENE was identified in 18 cases (45%) among the metastatic lymph nodes. 14 cases were classified as N1 and 26 as N2 according to AJCC 7th edition N category, incorporation of ENE resulted in 5 cases to be upstaged to form N1 to N2 nodal stage and 13 cases to upstaged from N2 to N3. **(Table.1)**

[Insert Table 1 **here]**

Table 1
Descriptive characteristics of the study cohort and the Association with lymph node metastasis:

Clinicopathological Variables		No. of patients	Lymph node metastasis		*p value*
		(N = 89)	pN0 (n = 49)	pN+ (n = 40)	
Age (years)	**Mean (SD)**	56.39 (12.63)	56.65 (13.14)	56.08 (12.13)	0.83[a]
	Min-Max	19.00–90.00	25.00–90.00	19.00–71.00	
Sex	**Male**	44 (49.44)	29 (59.18)	15 (37.50)	0.04*[b]
	Female	45 (50.56)	20 (40.82)	25 (62.50)	
Site	**Tongue**	41(46.07)	24(48.98)	17(42.5)	0.87[d]
	Buccal mucosa	21(23.6)	10(20.40)	11(27.50)	
	Retromolar area	12(13.48)	7(14.29)	5(12.5)	
	Floor of the mouth	4(4.49)	2(4.08)	2(5.00)	
	Mandible	4(4.49)	3(3.37)	1(2.50)	
	Hard palate	2(2.2)	1(2.04)	1(2.50)	
	Lower lip	2(2.2)	0(0.00)	2(5.00)	
	Gingiva	2(2.2)	1(2.04)	1(2.50)	
	Maxilla	1	1(2.04)	0(0.00)	
Tumor size (cm)	**Median (IQR)**	2.50 (2.00, 3.50)	3.00 (2.00, 3.50)	2.50 (2.00, 3.38)	0.68[c]
	Min-Max	1.00–10.00	1.00–10.00	1.00–6.00	
AJCC7th pT stage	**pT1 – pT2**	77 (86.52)	44 (89.80)	33 (82.50)	0.32[b]
	pT3 – pT4	12 (13.48)	5 (10.20)	7 (17.50)	

(SD) Standard Deviation, (IQR) Interquartile Range, (Min) Minimum, (Max) Maximum. (cm) centimeters, (mm) millimeters.

[a] Independent t test, [b] Chi-squared test, [c] Mann-Whitney U, [d] Chi-squared with Fisher's Exact adjustment

*Statistically significant at $p < 0.05$

AJCC8th pT stage	**pT1 – pT2**	56 (62.62)	33 (67.35)	23 (57.50)	0.34[b]
	pT3 – pT4	22 (37.08)	16 (32.65)	17 (42.50)	
DOI (mm)	**Median (IQR)**	8.00 (5.00, 13.00)	8.00 (4.00, 12.00)	10.00 (7.00, 15.25)	0.03*[a]
	Min-Max	2.00–20.00	2.00–20.00	3.00–20.00	
TB	**BD1**	45 (50.56)	35 (71.43)	10 (25.00)	< 0.001*[c]
	BD2	14 (15.73)	6 (12.24)	8 (20.00)	
	BD3	30 (33.71)	8 (16.33)	22 (55.00)	
PNI	**Not identified**	68 (76.40)	39 (79.59)	29 (72.50)	0.43[b]
	Present	21 (23.60)	10 (20.41)	11 (27.50)	
LVI	**Not identified**	71 (79.78)	42 (85.71)	29 (72.50)	0.12[b]
	Present	18 (20.22)	7 (14.29)	11 (27.50)	
LHR	**Strong**	28 (31.46)	20 (40.82)	8 (20.00)	0.01*[c]
	Intermediate	37 (41.57)	21 (42.86)	16 (40.00)	
	Limited	24 (26.97)	8 (16.33)	16 (40.00)	
WPOI	**1–3**	31 (34.83)	23 (46.94)	8 (20.00)	0.02*[c]
	4	34 (38.20)	15 (30.61)	19 (47.50)	
	5	24 (26.97)	11 (22.45)	13 (32.50)	
Neck dissection surgery	**Unilateral**	85 (95.51)	47 (95.92)	38 (95.00)	1.00[d]
	Bilateral	4 (4.49)	2 (4.08)	2 (5.00)	
AJCC7th pN stage	**pN1**			14(35.00)	
	pN2			26(65.00)	
AJCC8th pN stage	**pN1**			9(22.50)	
	pN2			18(45.00)	
	pN3			13(32.50)	

(SD) Standard Deviation, (IQR) Interquartile Range, (Min) Minimum, (Max) Maximum. (cm) centimeters, (mm) millimeters.

[a] Independent t test, [b] Chi-squared test, [c] Mann-Whitney U, [d] Chi-squared with Fisher's Exact adjustment

*Statistically significant at $p < 0.05$

Levels distribution of the metastatic LNs	**I**	21(52.5)
	II	20(50.00)
	III	12(30.00)
	IV	5(12.50)
	V	3(7.50)
ENE	**ENE -**	22(55.00)
	ENE +	18(45.00)

(SD) Standard Deviation, (IQR) Interquartile Range, (Min) Minimum, (Max) Maximum. (cm) centimeters, (mm) millimeters.

[a] Independent t test, [b] Chi-squared test, [c] Mann-Whitney U, [d] Chi-squared with Fisher's Exact adjustment

*Statistically significant at $p < 0.05$

Interobserver agreement

Worst pattern of invasion and LHR demonstrated good interobserver reproducibility (k = 0.74 both), while DOI, TB and PNI showed moderate agreement between the two observers (ICC = 0.6, k = 0.51 and 0.43 respectively). Fair agreement presented for LVI (k = 0.32).

Lymph node metastasis:

Association between histopathological parameters and LNM

A significant association was found between TB, LHR, WPOI and DOI, (p = < 0.001, 0.01, 0.02 and 0.03 respectively) with LNM. While pT stage, PNI and LVI were not significantly associated with lymph node metastasis. **(Table .1)**

Univariate analysis

Univariate logistic regression analysis was conducted to identify the predictor factors for lymph node metastasis. Only four parameters were significantly associated with lymph node metastasis; tumor budding (OR = 9.62, p value < 0.001, 95% CI 3.30-28.11 BD3 vs BD1), LHR (OR = 5, p value = 0.01, 95% CI 1.54–16.27 comparing limited LHR group with strong group), DOI was assessed as continuous variable (OR = 1.10, p value = 0.03, 95% CI 1.01–1.21) and WPOI (OR = 3.34, p value = 0.03, 95% CI 1.09–10.59). AJCC 7th pT stage, AJCC 8th pT stage, PNI and LVI were not statistically significant (OR = 1.87, p value = 0.32- OR = 1.52, p value = 0.34, OR = 1.48, p value = 0.43- OR = 2.28, p value = 0.13 respectively). **(Table.2)**

Multivariate analysis

Only the parameters that were statistically significant in the univariate analysis were included in the multivariate logistic regression analysis. Tumor budding was the only significant independent parameter for predicting lymph node metastasis after adjustment of other histopathological parameters (aOR = 7.63, p value < 0.001 95%CI 2.14–27.22 comparing high tumor budding with low tumor budding group). **(Table.2)**

[insert Table 2 **here]**

Table 2
Univariate and multivariate binary logistic regression models for the association between histopathological parameters and lymph node metastasis (N = 89):

Histopathological parameter		Univariate regression		Multivariable regression	
		OR (95% CI)	*p* value	aOR (95%CI)	*p* value
AJCC7th pT stage	**pT1-PT2**	Reference category		-	
	pT3-pT4	1.867(0.54–6.41)	0.32		
AJCC8th pT stage	**pT1-pT2**	Reference category		-	
	pT3-pT4	1.52(0.64–3.62)	0.34		
DOI (mm)		1.10 (1.01–1.21)	0.03*	1.07 (0.94–1.23)	0.29
TB	**BD1**	Reference category		Reference category	
	BD2	4.67 (1.31–16.63)	0.02*	3.91 (0.89–17.10)	0.07
	BD3	9.62 (3.30–28.11)	< 0.001*	7.63 (2.14–27.22)	0.002*
WPOI	**1–3**	Reference category		Reference category	
	4	3.64 (1.27–10.42)	0.02*	1.04 (0.26–4.20)	0.95
	5	3.40 (1.09–10.59)	0.03*	1.04 (0.23–4.20)	0.96
LHR	**Strong**	Reference category		Reference category	
	Intermediate	1.90 (0.67–5.42)	0.23	1.45 (0.42–5.07)	0.56
	Limited	5.00 (1.54–16.27)	0.01*	1.96 (0.41–9.25)	0.40
LVI	**Not identified**	Reference category		-	
	Present	2.28 (0.79–6.56)	0.13		
PNI	**Not identified**	Reference category		-	
	Present	1.48 (0.55–3.95)	0.43		

(OR) Unadjusted Odds ratio, (aOR) Adjusted Odds Ratio, (CI) Confidence Interval.

*Statistically significant at $p < 0.05$

DOI cut off

Receiver operating characteristics (ROC) curve analysis was conducted to determine the predictive cut-off value of DOI for lymph node metastasis (Fig. 8). Area under the curve (AUC) was 0.64 (95% CI 0.52–0.75, p value = 0.02). Based on Youden's index, a DOI cut-off value 7 mm showed the highest sum of specificity plus sensitivity for predicting lymph node metastasis with a sensitivity of 85% and specificity of 40.8%. The positive predictive value (PPV) and the negative predictive value (NPV) for this threshold

was 54% and 76.9% respectively. However, for patients with DOI < 7 mm the probability of lymph node metastasis is 23.1% which exceeds the commonly accepted 20% risk threshold for END in clinical practice(38, 39) **(Table.3,4)**.

For this reason, we additionally evaluated different cut-offs 4 mm and 5 mm, **(Table.3).** Using a DOI cut-off value of 4 mm, lymph node metastasis was identified in 1 of 9 cases within the DOI < 4 mm group compared with 39 of 80 cases in DOI ≥ 4 mm group. DOI cut-off value 4 mm show high sensitivity of 97% and the highest NPV 88.9% among assessed cut-offs **(Table.4)**. Indicating that 88.9% of patients that wouldn't have received a neck dissection, would truly be negative for the nodal metastasis. For patients with DOI < 4 mm the probability of lymph node metastasis is 11.1%.

Similarly, stratification using a 5 mm cut-off demonstrated lymph node metastasis in 4 of 18 cases (22.2%) in tumors with DOI < 5 mm compared with 36 of 71 cases (50.7%) in tumors with DOI ≥ 5 mm.

Univariate logistic regression analysis demonstrated significant associations between DOI-based categorization and lymph node metastasis **(Table.3)**. DOI cut-off values 5 mm was significantly associated with increased odds of nodal metastasis (OR = 3.60, 95% CI: 1.08–12.01, p = 0.037), while 7 mm cut off value demonstrated an odds ratio of 3.91 (95% CI: 1.38–11.04, p = 0.010). 4 mm cut off value showed a strong trend toward significance (OR = 7.61, 95% CI: 0.91–63.69, p = 0.061).

Figure (8) Receiver operating characteristic (ROC) curve for DOI cut-off value predicting LNM.

Table.3. Distribution of LNM according to DOI thresholds:

DOI cut-off		Total Cases	pN+ Cases	Metastasis probability	Univariate logistic regression	
					OR (95%CI)	p-value
4 mm	< 4 mm	9	1	**11.1%**	Reference category	
	≥ 4 mm	80	39	48.8%	7.61 (0.91–63.69)	0.061
5 mm	< 5 mm	18	4	22.2%	Reference category	
	≥ 5 mm	71	36	50.7%	3.60 (1.08–12.01)	0.037*
7 mm	< 7 mm	26	6	23.1%	Reference category	
	≥ 7 mm	63	34	54.0%	3.91 (1.38–11.04)	0.010*

(OR) odds ratio, (CI) confidence interval.

*Statistically significant at $p < 0.05$

Table.4 Diagnostic performance of DOI thresholds

DOI cut-off	Sensitivity	Specificity	PPV	NPV
4 mm	97.5%	18.4%	48.8%	88.9%
5 mm	90.0%	28.6%	50.7%	77.8%
7 mm	85.0%	40.8%	54.0%	76.9%

(PPV) positive predictive value, (NPV) negative predictive value.

Survival analysis

The follow-up data was available for only 46 patients (51.7%) of our cohort. 11 cases (23.91%) of them developed locoregional recurrence and 20 cases (43.48%) had deceased from the disease or any other cause. The mean follow up period for the total cohort was 19.24 months ranging between 1 and 108 months.

Univariate Cox proportional hazards regression was performed **(Table.5)**, lymphovascular invasion was a significant prognostic factor for LRRFS (HR = 3.55, 95%CI 0.29–4.08, p value = 0.04), while AJCC7th pT stage was a significant prognostic factor for OS (HR = 4.50. 95%CI 1.3- 15.58, p value = 0.02), however the other variables demonstrated increased hazard for LRR and OS but these didn't reach statistically significant values. Multivariate Cox regression analysis was not conducted as the number of events was insufficient.

Kaplan Meier survival analysis was performed to compare overall survival (OS) and locoregional recurrence free survival (LRRFS) among groups of the histopathological parameters (Fig. 9, 10) and demonstrated significantly reduced LRRFS in patients with lymphovascular invasion compared to LVI negative tumors (Log rank p value = 0.031), but not OS. AJCC7th pT stage was associated significantly with reduced both OS and LRRFS (Log rank p value = 0.01 and 0.046 respectively).

Positive ENE, High tumor budding, WPOI-5, limited LHR show earlier locoregional recurrence and shorter overall survival. But this didn't reach statistically significant association.

[insert Table 5 **here]**

Figure (9) Kaplan–Meier survival curves comparing locoregional recurrence free survival (LRRFS) in patients' groups based on histopathological parameters.

Figure (10) Kaplan–Meier survival curves comparing overall survival (OS) in patients' groups based on histopathological parameters.

Discussion

Since lymphatic spread to regional lymph node is common, neck management is a cornerstone treatment strategy in OSCC with its associated esthetics and functional morbidity. The key challenge is, selecting patients who truly benefit from END while sparing those unlikely to harbor metastasis (40). The present study comprehensively evaluated the prognostic significance of both established and emerging histopathological parameters in OSCC and their utility in predicting lymph node metastasis, highlighting the value of histopathological features that extend beyond conventional staging and support their integration into routine standardized reporting systems to enhance clinical decision making.

DOI has emerged as one of the most important histopathological predictors of cervical lymph node metastasis in OSCC, its incorporation into the T category of the 8th edition of the AJCC staging system in 2017 has important therapeutic implications (14, 41). This results in stage migration of a considerable proportion of patients from early to more advanced disease categories (42–44). In our cohort, 21 cases (23%) were upstaged form early (T1-T2) to advanced (T3-T4) stages. Such stage migration may significantly influence treatment planning by shifting patients toward treatment protocols that include END and/or adjuvant radiotherapy (8). DOI was significantly associated with LNM when analysed as a continuous variable, with each one mm additional DOI, there is an increase in the odds of nodal metastasis by approximately 1.1 times. Nevertheless, its incorporation into the T stage category according to AJCC 8th edition didn't improve the prognostic discrimination in our cohort either in the LNM or the survival outcomes. The AJCC 7th edition pT stage showed a significant association with overall survival. This finding may reflect the heterogeneous outcomes of patients upstaged solely based on DOI, which reduced the prognostic separation between early- and advanced-stage disease categories.

In addition to its incorporation into tumor staging, DOI has gained considerable attention as a tool for guiding neck management decisions (8, 39). However, determining the optimal DOI cut off for END remains controversial, with reported cut off values ranging from 2 mm to 10 mm. Although most studies and NCCN recommendations support considering END at 4mm DOI (6, 45–47), Our ROC analysis identified 7 mm as an optimal DOI cut off value for LNM. This finding is comparable to the results of Tam et al. who reported a DOI cut off 7.25 mm in a large cohort study of oral tongue squamous cell carcinoma (OTSCC) (48). Tumors with DOI < 4 mm in our cohort had an 11.1% probability of LNM and high negative predictive value (NPV) of 88.9%. This supports the superiority of the 4mm cut off in identifying the low-risk patients for LNM who may potentially avoid unnecessary neck dissection. Indeed, a small portion of tumors with DOI < 4 mm still developed nodal metastasis. Whereas some deeply invasive tumors remained node negative explained by the low specificity of this cut off value. These findings collectively emphasize that DOI is significantly correlated with LNM and remains a critical parameter for guiding surgical decisions, but it likely represents only one aspect of tumor aggressiveness. Therefore, additional histopathological parameters reflecting the biological behaviour of the invasive front may provide complementary prognostic information.

Tumor budding at the invasive front is a well-established adverse prognostic factor in colorectal(24, 25), lung, pancreatic and esophageal cancers (26, 27). Consequently, It has been incorporated as a recommended item into the reporting protocols of colorectal cancer by both the College of American

Pathologists (CAP) and International Collaboration On Cancer Reporting (ICCR) (49, 50). In OSCC, tumor budding has gained increasing attention over recent years and remains an active area of investigation. Numerous studies have evaluated the prognostic significance of TB in OSCC and supported its value as a predictor for LNM (51–54). In a recent large meta-analysis involving 142 studies, Alqutub et al. recommended routine reporting of TB whenever possible in final histopathological examination (6). Similarly, karjol et al. and Almangush et al demonstrated a significant association between high TB and LNM (54, 55), and yet it is still not included till present in reporting systems of OSCC.

In our study, multivariate analysis for predicting nodal metastasis demonstrated that TB is an independent significant predictor of nodal metastasis after adjustment for DOI and other clinicopathological variables. Patients with high tumor budding demonstrated 7.6-fold higher odds of developing nodal metastasis compared with those exhibiting low tumor budding. Furthermore, univariate cox regression revealed an approximately two-fold increased hazard of death and one-fold increased risk of LRR in the high tumor budding group. Kaplan Meier survival curves also revealed earlier LRR and shorter overall survival among tumors with high TB despite being non-significant.

Different methods, cut offs and tier systems have been proposed for TB assessment (56). The ITBCC for colorectal cancer in 2016 established a standardized method for tumor budding assessment (31). Our findings precisely mirror those of Salama et al and Shimizu et al. who also assessed TB according to ITBCC recommendations and furthermore identified TB as the only significant independent predictor factor for LNM in multivariate analysis after adjustment for DOI, WPOI and other established histopathological factors (57, 58). Although most OSCC studies have utilized the two-tier system proposed by Wang et a (52), Bjerkli et al. suggested that the three-tier system has superior prognostic stratification (22). Furthermore, Xie et al. demonstrated that TB was correlated significantly with both LNM and poor survival in OTSCC based on ITBCC assessment and stated it as a reliable, reproducible, and practicable TB scoring system that should be incorporated into reporting OSCC (59).

The superiority of TB as a predictive marker likely stems from its direct biological representation of the epithelial-mesenchymal transition (EMT) (60, 61). During EMT, tumor cells lose cellular adhesion (e.g., decreased E-cadherin) and gain migratory capacity (e.g., increased vimentin), which are the fundamental first steps required for lymphatic invasion and metastasis (52, 62). Because TB captures this active, non-cohesive invasive movement at the cellular level, it provides a more precise indication of metastatic potential than broader architectural measurements like DOI or WPOI (23, 61, 63).

Worst pattern of invasion represents another form of architectural aggressiveness of the tumor at the invasive front. Despite its recognized prognostic importance, the College of American Pathologists (CAP) considered it as a non-core optional parameter and recommends documenting specifically only WPOI-5 (49). We observed a significant association between aggressive invasion patterns and LNM. In univariate analysis, both WPOI-4 and WPOI-5 were associated with significantly increased odds of nodal metastasis compared to non-aggressive WPOI 1–3, with odds ratios of 3.6 and 3.4 respectively. In the univariate cox regression analysis, WPOI-5 showed 1.5-fold increased hazard of LRR (p.= 0.6). These

findings are consistent with previous studies establishing that aggressive invasive patterns, particularly WPOI-4 and WPOI-5, as reliable markers of cellular dissociation and poor clinical outcomes(53, 64, 65).

Interestingly, WPOI-4 demonstrated a slightly stronger association with LNM than WPOI-5 and represented the most common aggressive invasion pattern encountered in our study population. These findings support previous observations by Parekh et al., who emphasized that WPOI-4 carries prognostic significance comparable to WPOI-5 (66). Therefore, this pattern would be better evaluated and reported as a separate entity rather than being grouped with less aggressive invasion patterns.

Chang et al. in a recent study proposed a modified WPOI and tumor budding model in which he grouped WPOI-4 with the less aggressive patterns (WPOI 1–3) (70). Our findings ,that shows a distinction between WPOI-4 and WPOI 1–3 ,raise important considerations regarding the proposed reclassification (66). Morais et al. also found that the classical WPOI system provided superior discriminatory ability across all of OSCC stages (67). On the other hand, Almangush et al. stated the importance of the modified WPOI in identifying high risk cases, though their study was limited to early SCC (68).

Despite the strong prognostic performance of WPOI in our univariate analysis, WPOI didn't retain independent significance in the multivariate analysis. A similar observation was reported by Csizmazia et al. where WPOI lost significance after adjustment for other histopathological parameters (69). We return this to the biological overlap between WPOI and TB, as both parameters reflect cell dissociation and invasive behaviour at the advancing tumor front. Indeed, tumors exhibiting WPOI-4 and WPOI-5 showed a markedly higher frequency of high TB compared with WPOI 1–3. In our cohort sample, more than half of WPOI-4 tumors (52.9%) were classified as BD3, whereas only 12.9% of tumors with WPOI 1–3 demonstrated BD3.

Tumor microenvironment (TME) is a complex ecosystem comprises diversity of immune cells surrounding cancerous epithelial cells. Lymphocytic host response reflects the interaction between tumor cells and host immune system at the invasive front (70). In our cohort, we found that LHR significantly correlates with LNM in the univariate analysis. Tumors exhibiting little or absent lymphocytic response at the invasive front were associated with a fivefold increased risk of nodal metastasis compared to tumors showing strong LHR. Furthermore, our survival analysis also showed consistent trend toward poor OS and LRR. These findings support that an effective local immune response may contribute to limiting tumor dissemination and disease progression (5, 71).

Although it is not currently included in the college of American pathologist (CAP) standard reporting protocol for OSCC or any other alternative item reflects the immune related properties, growing interest in the TME has renewed attention to immune related histopathological markers (66, 72, 73). Lymphocytic host response may represent an available simple morphologic indicator for immune activity within TME, that may aid in patient's stratification for immunotherapeutic approaches.

Lymphovascular invasion (LVI) represents a critical step in the metastatic cascade, reflecting the ability of tumor cells to penetrate endothelial lined lymphatic or vascular channels and disseminate beyond the

primary tumor site (8, 36, 74).

In the present study, LVI demonstrated an odd of 2.2 for nodal metastasis on univariate analysis. Although LVI was not significantly associated with nodal metastasis in our cohort, it demonstrated a significant association with LRR on univariate Cox regression analysis. This finding is consistent with the biological role of LVI as an indicator of tumor dissemination and aligns with previous reports linking LVI to adverse oncologic outcomes. Shaikh et al (75) identified LVI as the only parameter independently associated with LRR, while Cassidy et al. reported worse locoregional control and OS among node-negative oral tongue cancer patients with LVI (76). These observations suggest that LVI may reflect an aggressive tumor phenotype prone to disease recurrence even if its association with nodal metastasis is not independently demonstrable.

The literature regarding the significance of LVI in OSCC is conflicting. Several studies have identified LVI as an important predictor of nodal metastasis and poor prognosis. Huang et al. emphasized that invasion of lymphovascular channels is a crucial biological event in the metastatic process and demonstrated the prognostic value of LVI for predicting cervical LNM in early-stage OSCC (35). Also, the recent comprehensive meta-analysis by Alqutub et al., underscored the clinical importance of LVI as a predictor of LNM in oral cavity squamous cell carcinoma (6).

On the contrary, Kane et al. failed to demonstrate a significant relationship between LVI and LNM in early-stage OSCC (11). While Narayana Subramaniam et al. noted that the prognostic significance of LVI remains controversial (77). Such discrepancies may reflect biological heterogeneity among OSCC cohorts, differences in patient selection and study design, and variability in histopathological assessment of LVI.

In our study, the presence of PNI was associated with 1.48-fold increased odds of lymph node metastasis with shorter OS and earlier LRR on Kaplan–Meier analysis; however, neither of these findings reached statistical significance. This further support the concept that traditional dichotomous evaluation (present/absent), as applied in our study, often fails to show an independent, statistically significant impact on survival. Emerging evidence suggests that specific PNI characteristics, particularly multifocal involvement and invasion of large nerves (≥ 1 mm), are more strongly associated with nodal metastasis, local recurrence, and poorer survival (78, 79). This concept is potentiated by recent reviews by Timothy Liu et al., Chatzistefanou et al., and the systematic review by Bur et al. They proposed that only high-risk forms of PNI derive substantial benefit from adjuvant radiotherapy, whereas patients with isolated low-risk or unifocal PNI may be overtreated (80–82). While current guidelines continue to consider PNI an indication for adjuvant therapy, a more risk-adapted approach may improve patient stratification and reduce unnecessary treatment-related morbidity (78, 79).

Extranodal extension represents an established prognostic factor of in OSCC which is responsible for 50% reduction in OS and disease-specific survival (DSS) among patients with pENE (18, 83, 84). Owing to its adverse prognostic implications, pENE is widely regarded as an indication for treatment intensification in HNSCC. A pooled subset analysis of the landmark EORTC 22931 and RTOG 9501 trials

established the benefit of adding concurrent high-dose cisplatin to adjuvant radiotherapy in patients with pENE and/or positive surgical margins (8, 85, 86).

In the same context, we found that patients with positive ENE exhibited an approximately 2-fold increased hazard of LRR and mortality compared to ENE-negative patients. However, these associations did not reach statistical significance, likely owing to the limited number of recurrence and death events observed among our ENE-positive cases. Furthermore, the incorporation of ENE in the AJCC 8th edition as a part of N category also results in stage migration similar to the effect observed for the DOI.Eighteen cases (20.2%) of our cohort were upstaged to a higher nodal category (N2 or N3), leading to their reclassification as stage IV diseases, 15 of which were assigned to N3b category due to the presence of ENE.

Evaluation of these parameters using digital pathology reflects the ongoing shift towards technology-assisted pathology and may facilitate wider implementation of prognostic markers in routine diagnostic workflows, specially in the era of digital pathology that continuous to evolve rapidly, driven by advances in whole-slide imaging, image analysis software, and computational pathology.

Future research should focus on developing and validating robust artificial intelligence algorithms for the assessment of the histopathological risk factors. This enables more standardized, reproducible, and efficient reporting while supporting pathologists, surgeons, and oncologists in evidence-based clinical decision-making. Moreover, the integration of digital pathology with clinicopathological and molecular data may facilitate the development of comprehensive risk prediction models for personalized patient management.

The present study has some limitations; its retrospective design and relatively limited sample size. In addition, a substantial proportion of patients lacked complete follow-up information. Inconsistent data entry across departments, difficulties in patient tracking, and incomplete documentation of causes of death restricted the availability of reliable long-term outcome data and precluded a comprehensive analysis of disease-specific survival. Furthermore, the relatively low number of recurrence and death events reduced the statistical power of survival analyses and may have contributed to the lack of statistically significant associations between certain histopathological parameters and survival outcomes.

Another limitation is the heterogeneity of the study cohort, which included both early- and advanced-stage OSCC. While the inclusion of tumors across a broad disease spectrum enhances the generalizability of the findings and allows assessment of histopathological risk factors over a wide range of tumor aggressiveness, it may also introduce confounding effects related to differences in tumor biology, treatment modalities, and clinical behavior between stages. Nevertheless, this heterogeneous cohort may better reflect real-world clinical practice.

Despite these limitations, to the best of our knowledge, this is the first study to comprehensively evaluate multiple histopathological risk factors using whole slide imaging in a sample of Egyptian

population with oral squamous cell carcinoma and investigate their associations with lymph node metastasis and survival outcomes. These findings provide valuable data from an underrepresented population and contribute to the growing evidence supporting the prognostic significance of histopathological parameters in oral squamous cell carcinoma.

Conclusion

In conclusion, this study demonstrates that tumor budding is a robust and independent predictor of lymph node metastasis in oral squamous cell carcinoma and supports its incorporation into routine histopathological reporting and risk stratification protocols. Our findings also highlight the prognostic relevance of WPOI-4 and limited lymphocytic host response, suggesting that these parameters warrant greater consideration as distinct pathological indicators of tumor aggressiveness. Furthermore, our results support the clinical utility of depth of invasion in guiding management decisions and suggest that a subset of patients may be spared unnecessary elective neck dissection thereby reducing treatment-related morbidity. The findings also emphasize the importance of careful interpretation of contemporary staging systems, particularly when stage migration may influence decisions regarding adjuvant therapy

Beyond the identification of prognostic markers, the ultimate aim is to ensure that every patient receives neither more nor less treatment than necessary, but the most appropriate care to achieve the best possible outcome and quality of life.

Future multicentric prospective studies with larger cohorts and comprehensive long-term follow-up are required to further validate the predictive and prognostic values of these histopathological markers and to establish standardized, reproducible methodologies for their routine assessment. Ultimately, integration of digital pathology, whole-slide imaging, and artificial intelligence-assisted image analysis represents the next frontier in head and neck pathology, offering unprecedented opportunities to enhance diagnostic precision, improve reproducibility, and support personalized clinical decision-making.

Abbreviations

aHR
Adjusted hazard ratio
AJCC
American Joint Committee on Cancer
aOR
Adjusted odds ratio
AUC
Area under the curve
CI

Confidence intervals
DOI
Depth of invasion
END
Elective neck dissection
ENE
Extranodal extension
HR
Hazard ratio
ICC
Intraclass correlation coefficient
ICCR
International Collaboration On Cancer Reporting
IQR
Interquartile range
ITBCC
International Tumor Budding Consensus Conference
LHR
Lymphocytic host response
LRRFS
Locoregional recurrence free survival
LNM
Lymph node metastasis
LVI
Lymphovascular invasion
NCCN
National Comprehensive Cancer Network
OR
Odds ratio
OS
Overall survival
OSCC
Oral squamous cell carcinoma
PNI
Perineural invasion
ROC
Receiver operating characteristic
SD
Standard deviation
TB

Tumor budding
TME
Tumor microenvironment
UICC
Union for International Cancer Control
WPOI
Worst pattern of invasion
WSI
Whole slide image

Declarations

- **Ethics approval and consent to participate**

This retrospective study protocol was reviewed and approved by Research Ethics Committee, Faculty of Dentistry, Alexandria University, Institutional Review Board (IRB) under approval number (IRB 001056-IORG 0008839). Ethical no. 1062-04/2025. All procedures performed in our study involving human data and material were in accordance with the Declaration of Helsinki. Our study utilized retrospective histopathological slides and clinical reports from the archives of Alexandria University, Informed consent was waived by the Research Ethics Committee, Faculty of Dentistry, Alexandria University and a previous informed consents for the use of Patients' data and material in research were obtained from all patients before their initial surgery, as part of the standard institutional procedure in Alexandria main hospital.

- **Consent for publication**

Not Applicable.

- **Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests

- **Funding**

Not applicable

- **Author contributions**

N.R. was responsible for conceptualization, data curation, investigation, statistical analysis, and writing the original manuscript. M.N. participated in the investigations, validation, supervision and critical

revision of the manuscript. H.S. was responsible for provision of access to records, supervision of the study and revision of the manuscript. G.K. and M.M. were responsible for validations, provision of access to follow-up records, supervision of the study and revision of the manuscript. O.R was responsible for project administration, validation, supervision, resources and critical revision of the manuscript. All authors read and approved the final manuscript.

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Table 5

Table 5 is available in the Supplementary Files section.

Figures

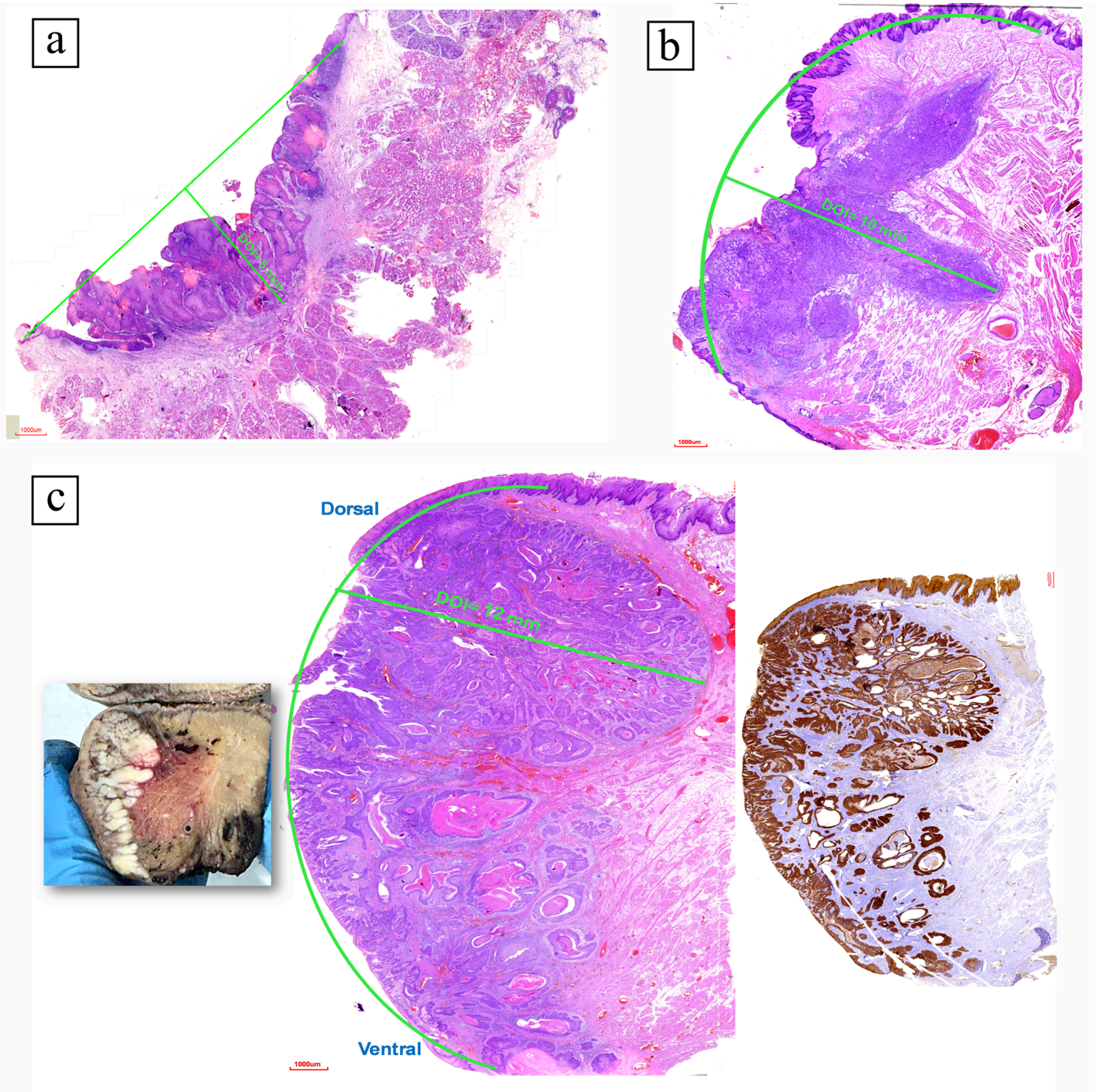


Figure 1

Whole slide images for OSCC. **(a)** An ulcer in the buccal mucosa, a straight line was drawn from the normal mucosa on both sides, and a perpendicular line was dropped to the deepest point of invasion. **(b-c)** OSCC related to the lateral border of the tongue, a convex arcuate line was drawn instead of straight horizontal line to reconstruct the natural contour of the tongue. **(c)** Gross examination (left picture) showing the convex natural contour of the tongue, H&E slide (middle picture), and pan cytokeratin immunohistochemical staining (right picture).

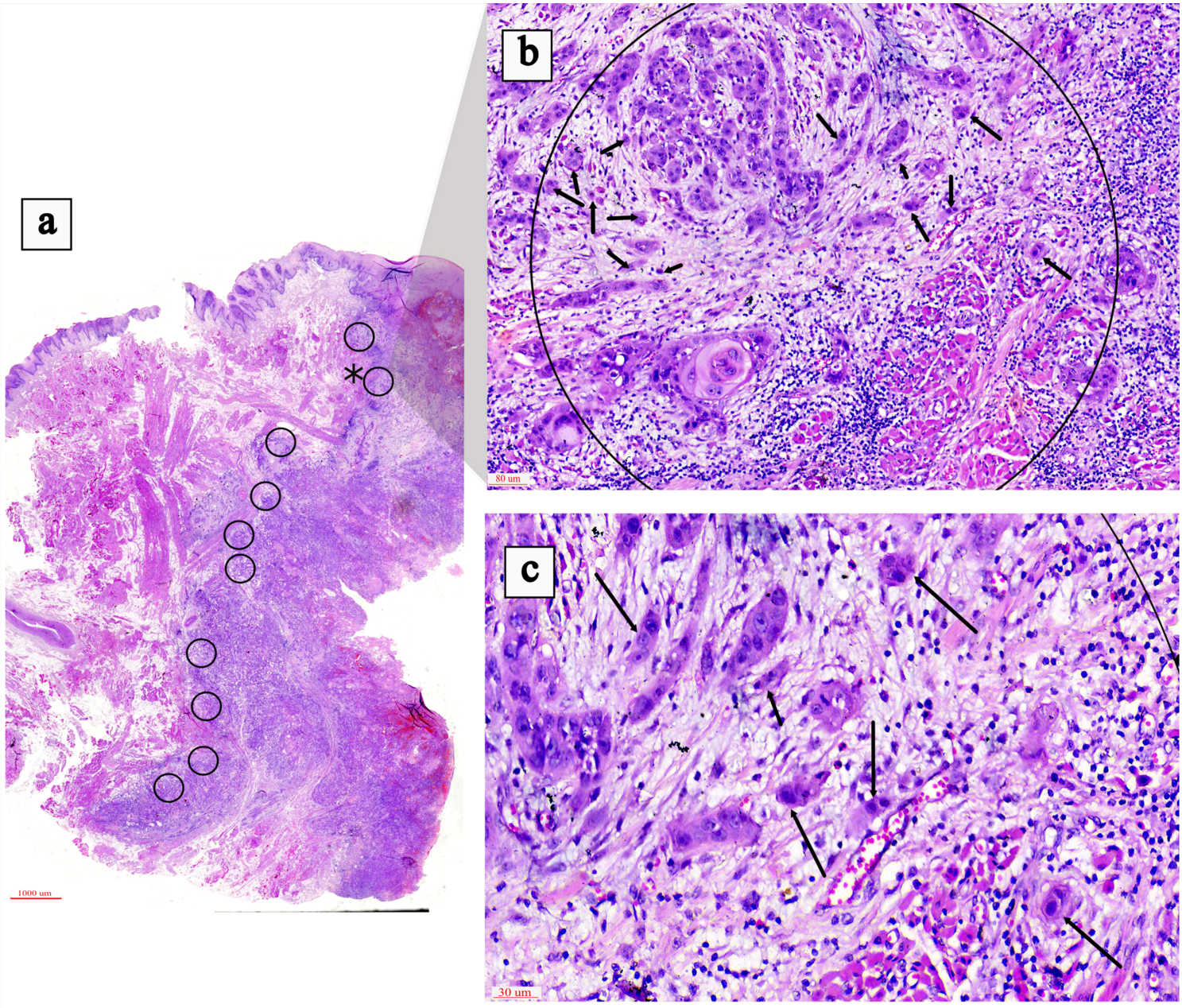


Figure 2

A case with an ulcer at the left lateral border of the tongue. (a) 10 circles (0.785 mm^2 each) were drawn at the invasive front of the tumor. **(b)** High tumor budding BD3; > 10 buds in the hot spot chosen circle (*), limited LHR and WPOI-4. **(c)** High magnification shows tumor buds as a single cell up to 4 cells (black arrows).

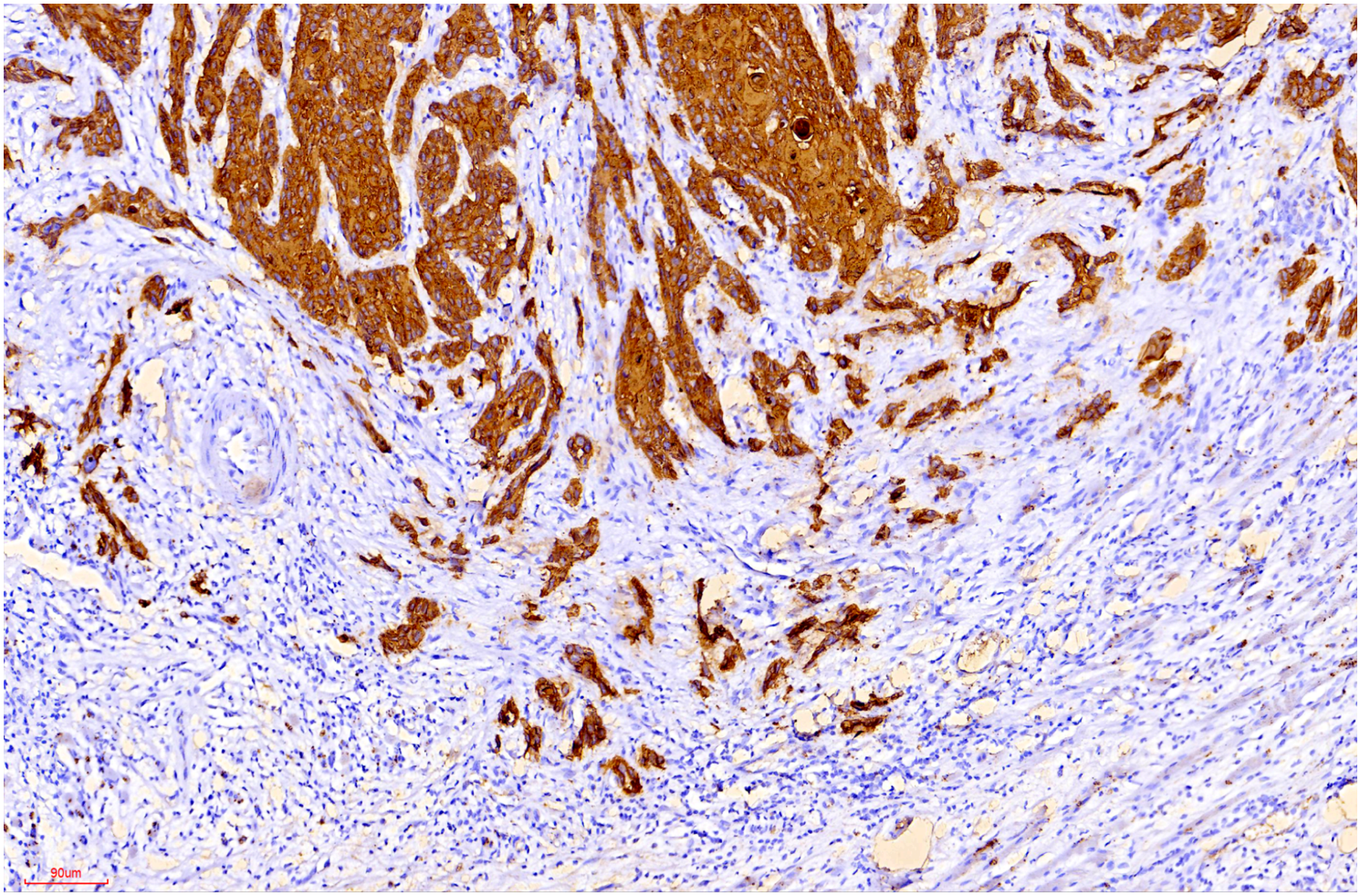


Figure 3

Pan-cytokeratin immunohistochemical staining demonstrated high tumor budding (BD3) at the invasive front of the tumor.

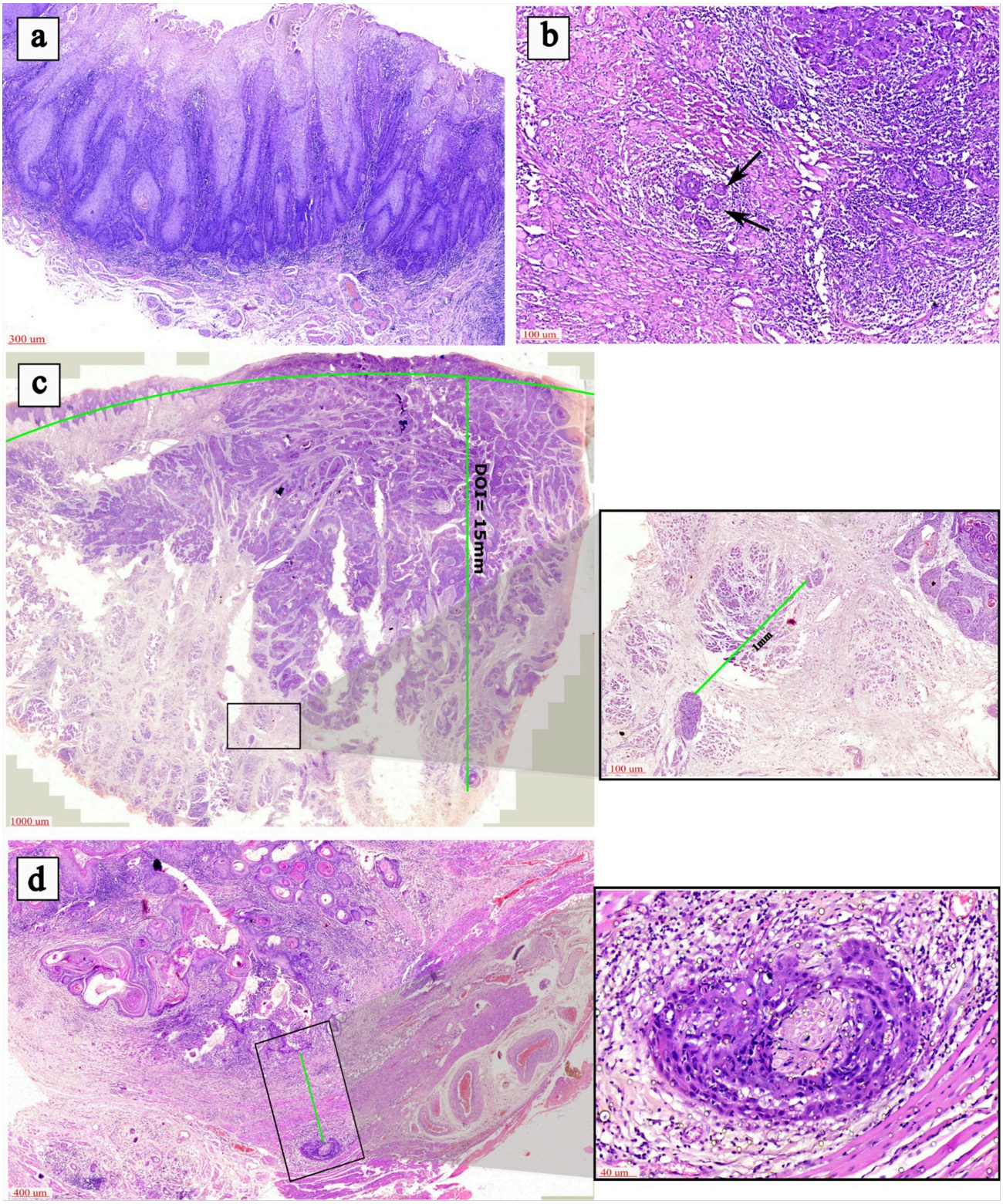


Figure 4

(**a)** WPOI-2; finger like invasion pattern. **(b)** WPOI-4; convincingly separate invasive island less than 15 cell (black arrows). **(c)** A case with skip metastasis to level IV shows WPOI-5, limited LHR and DOI 15 mm. **(d)** WPOI-5 represented as perineural invasion away >1 mm from the main tumor.

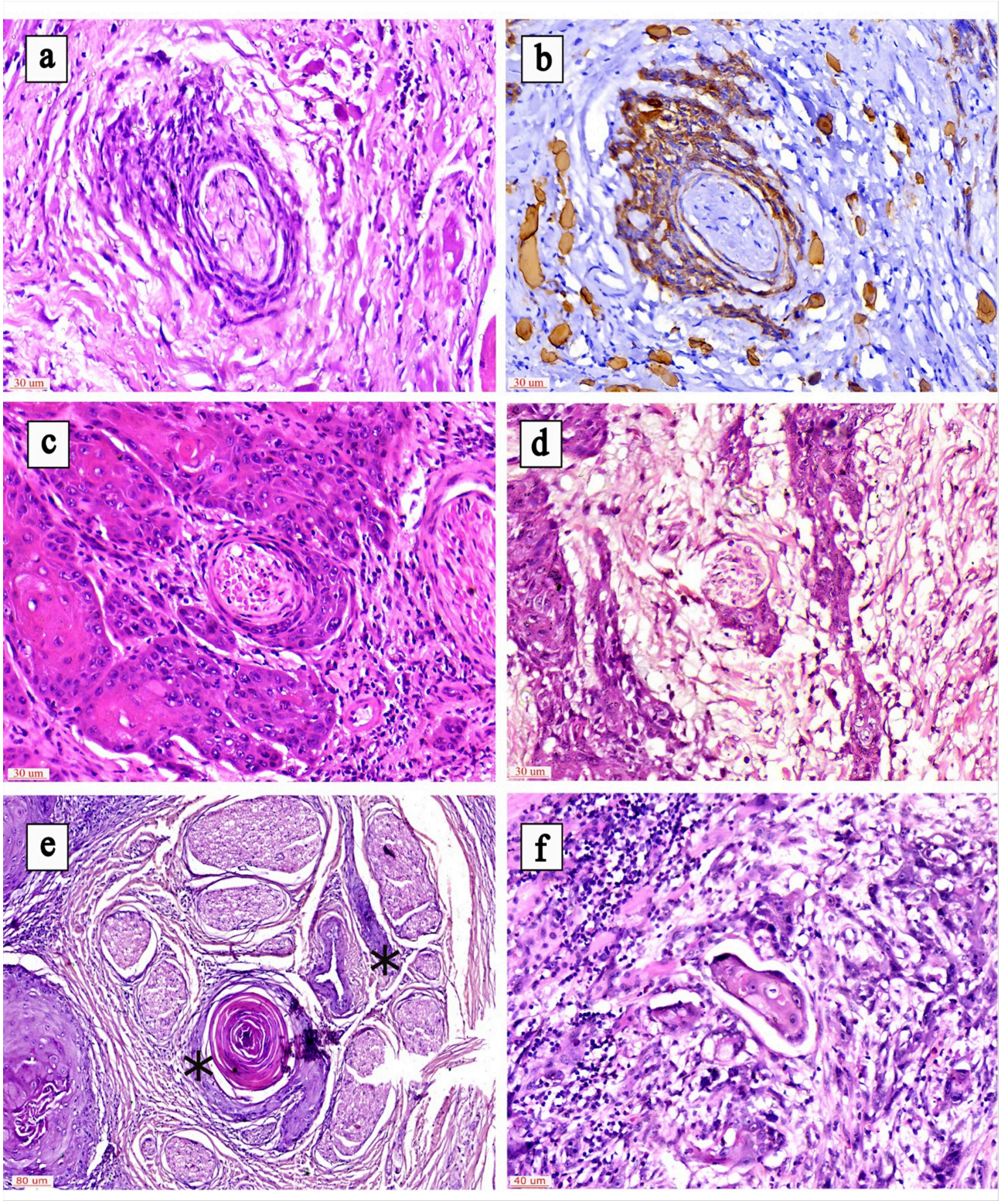


Figure 5

Perineural and lymphovascular invasion in OSCC. (a-b) H&E and pan cytokeratin immunohistochemical staining show total encirclement of the nerve by malignant epithelial cells. **(c-d)** Encirclement of 33% or more the nerve diameter. **(e)** Intraneural PNI, malignant epithelial cells (*) invading large nerve epineurium layer. **(f)** Embolus of malignant cells within an endothelial lined vessel.

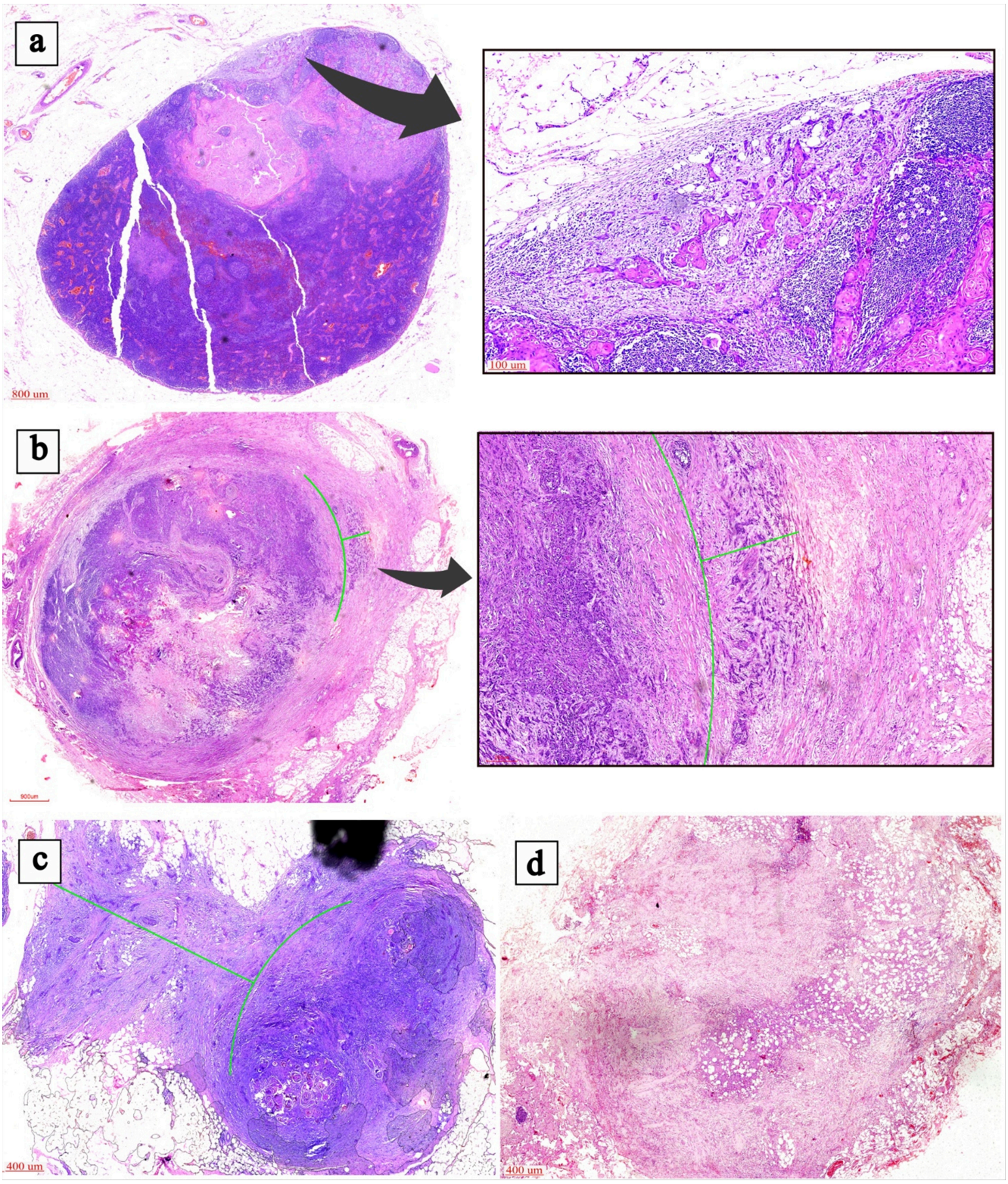


Figure 6

Positive ENE metastatic lymph nodes. (a) Minor ENE, metastatic deposits interact with perinodal fat tissue. **(b)** Minor ENE, metastatic deposit presents within the duplicated capsule. **(c)** Major ENE >2 mm. **(d)** Soft tissue deposit considered as major ENE.

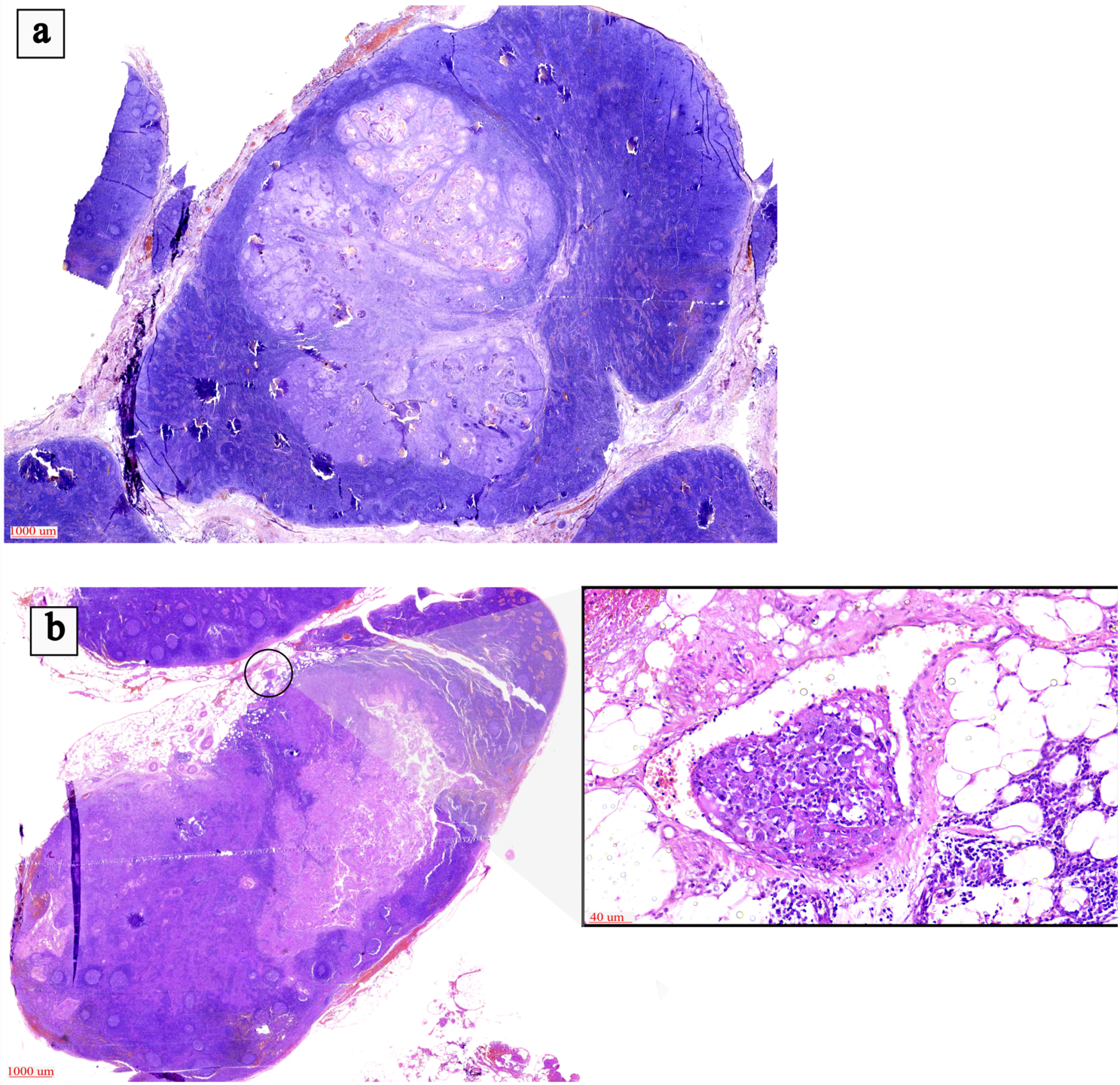


Figure 7

Negative ENE metastatic lymph nodes. (a) Metastatic deposits confined within the lymph node. **(b)** Lymphovascular embolus of malignant epithelium cells within a vessel in the lymph node helium considered as negative ENE.

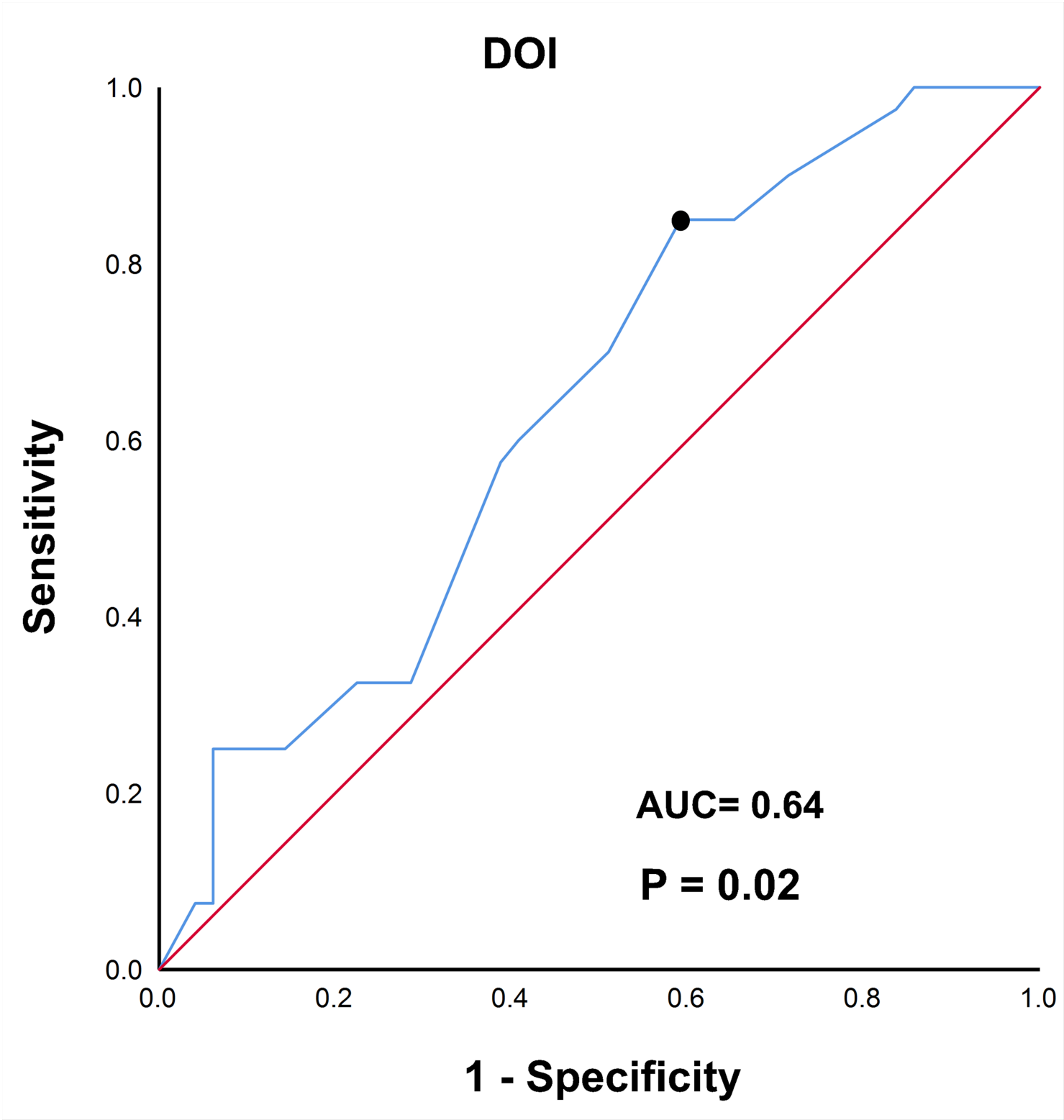


Figure 8

Receiver operating characteristic (ROC) curve for DOI cut-off value predicting LNM.

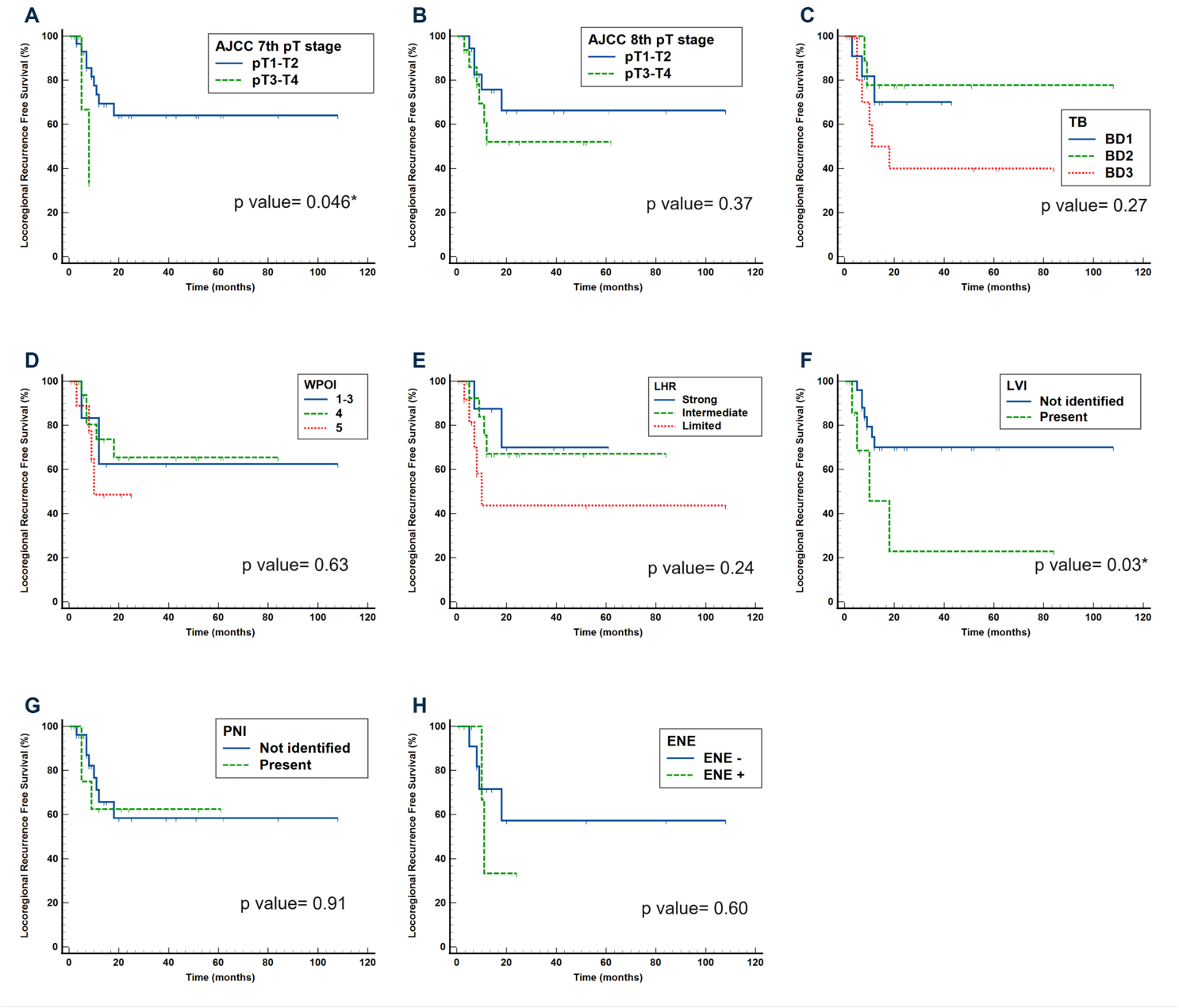


Figure 9

Kaplan–Meier survival curves comparing locoregional recurrence free survival (LRRFS) in patients' groups based on histopathological parameters

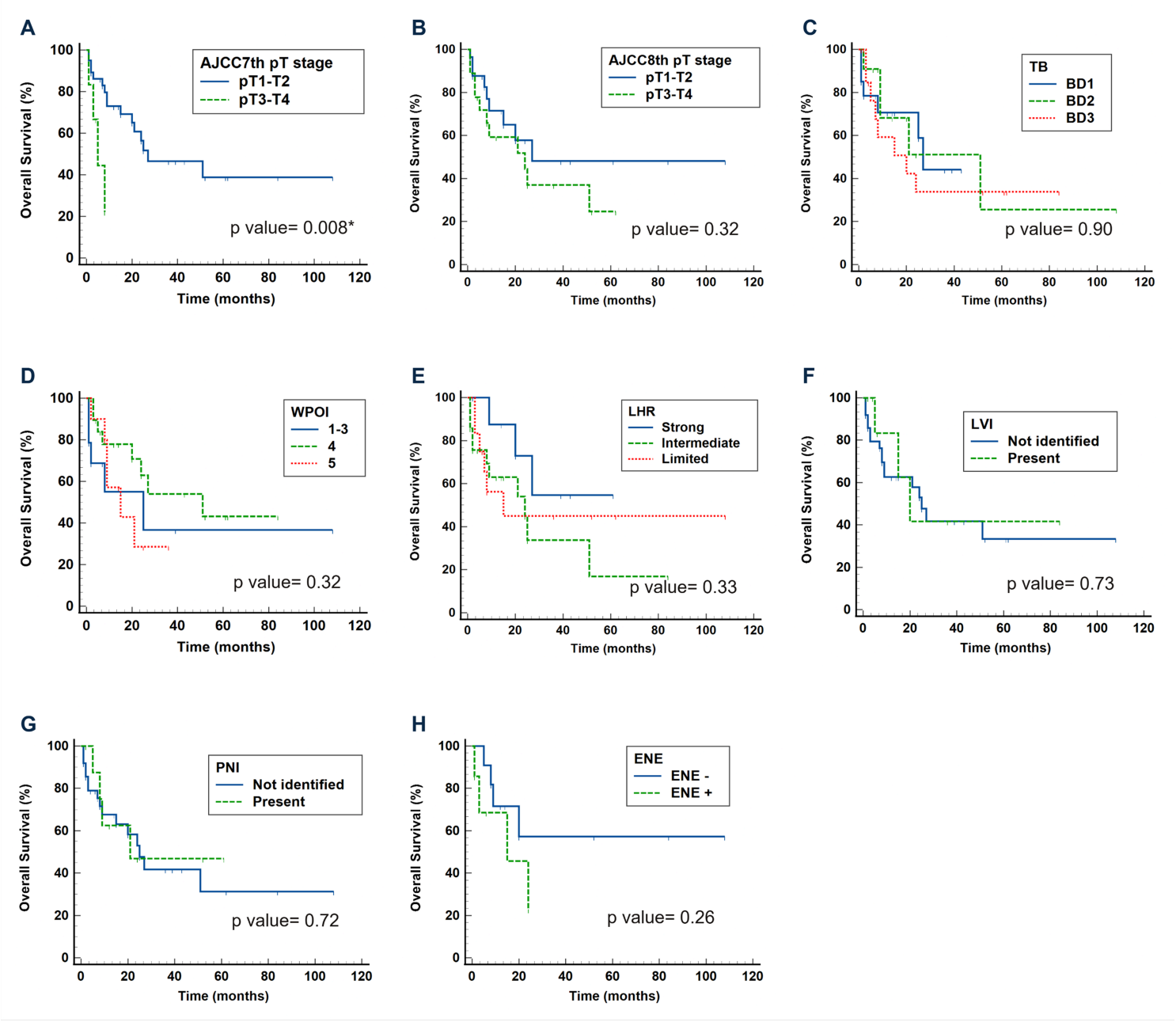


Figure 10

Kaplan–Meier survival curves comparing overall survival (OS) in patients' groups based on histopathological parameters

Supplementary Files

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- Table5.docx