

Original Article

Development and validation of a prediction model for lymph node metastasis in oral squamous cell carcinoma using serum biomarkers

Li Fan

Department of Head and Neck Surgery, Shanxi Provincial Cancer Hospital, No. 3, Zhigongxin Street, Xinghualing District, Taiyuan, 030000, Shanxi, China

Received August 12, 2025; Accepted November 4, 2025; Epub November 15, 2025; Published November 30, 2025

Abstract: Objective: To investigate the predictive value of serum soluble T-cell immunoglobulin and mucin domain-3 (sTIM-3), transforming growth factor-beta 1 (TGF- β 1), and vasohibin-1 in the lymphatic metastasis of oral squamous cell carcinoma (OSCC). Methods: A total of 220 OSCC patients admitted to Shanxi Provincial Cancer Hospital between January 2022 and December 2024 were included in this retrospective study. The patients were divided into training and validation sets at a 7:3 ratio (154 and 66 patients, respectively). Baseline characteristics, blood test results, and tumor marker levels were compared between the two groups. Predictors were screened, and column-line graphical models were constructed using Least Absolute and Residual Selection Operator (LASSO) regression and multifactorial logistic regression. The performance of the model was then evaluated using ROC curves, calibration curves, and decision curve analysis. Results: LASSO regression identified the following variables as predictors: clinical stage, tumor diameter, squamous cell carcinoma antigen (SCC-Ag), and carcinoembryonic antigen, sTIM-3, TGF- β 1, and vasohibin-1. Multifactorial logistic regression analysis revealed that clinical stage, SCC-Ag, sTIM-3, TGF- β 1, and vasohibin-1 were independent predictors of lymphatic metastasis. The AUC of the nomogram model was 0.868 in the training set and 0.863 in the validation set, indicating strong discriminatory ability. Calibration curves showed good agreement between predicted and actual values, with *p*-values for goodness of fit of 0.865 (training set) and 0.872 (validation set). Decision curve analysis demonstrated significant clinical benefit, with maximum benefit rates of 39.41% in the training set and 37.80% in the validation set. Conclusion: sTIM-3, TGF- β 1, and vasohibin-1, along with clinical stage and SCC-Ag, are independent predictors of lymph node metastasis in OSCC patients. The risk prediction model based on these variables demonstrates strong predictive ability.

Keywords: Oral squamous cell carcinoma, lymph node metastasis, tumor markers, logistic regression, nomogram

Introduction

Oral squamous cell carcinoma (OSCC), the most common malignant tumor of the head and neck, accounts for over 90% of oral malignancies. It is characterized by aggressive behavior, rapid growth, high recurrence rate, and poor prognosis [1]. According to the latest World Health Organization statistics, there are over 300,000 new OSCC cases globally each year, with incidence rates among the top ten malignant tumors in high-incidence regions, such as Southeast Asia [2]. Currently, OSCC treatment involves a multidisciplinary approach, including surgical resection combined with radiotherapy, chemotherapy, and immunother-

apy. Surgical intervention alters local anatomy and facial appearance, affecting functions such as chewing, swallowing, and speech. Radiotherapy and chemotherapy often have severe side effects, while immunotherapy is expensive. These factors contribute to poor patient adherence to treatment and suboptimal prognosis. A major challenge is that current treatment options are only partially effective for advanced or metastatic OSCC, with drug resistance and recurrence often complicating treatment and limiting long-term survival benefits [3, 4]. Lymph node metastasis is a key factor influencing treatment outcomes and patient prognosis in OSCC [5]. Clinical studies show that patients with OSCC who develop lymph node

metastasis have significantly higher recurrence rates after surgery, lower five-year survival rates, and diminished quality of life [6]. However, early diagnosis of lymph node metastasis in OSCC lacks effective clinical indicators with high specificity and sensitivity, leading to disease progression. Therefore, identifying reliable markers that can predict early lymph node metastasis in OSCC is crucial for improving survival rates and quality of life.

Pathological examination remains the gold standard for diagnosis due to its high accuracy. However, its invasive nature causes pain and trauma to the patient and limits its use for pre-operative dynamic monitoring, thus failing to reflect timely changes in disease progression [7]. In recent years, advancements in molecular biology and immunology have made serum tumor markers a popular focus for predicting tumor metastasis, offering the advantages of convenience, minimal invasiveness, and repeatability [8]. Various cytokines and immunoregulatory molecules play significant roles in tumor invasion and metastasis. For instance, soluble T-cell immunoglobulin mucin 3 (sTIM-3) accelerates tumor progression by suppressing the immune response and allowing tumor cells to evade immune surveillance [9]. Elevated sTIM-3 levels are associated with poor prognosis in several cancers, including hepatocellular carcinoma, renal carcinoma, and breast cancer [10, 11]. Transforming growth factor-beta 1 (TGF- β 1) contributes to tumor microenvironment remodeling, promoting angiogenesis and metastasis formation [12]. While TGF- β 1 has anti-proliferative effects in the early stages of OSCC, its immunosuppressive effects predominate in later stages, acting as a tumor promoter [13]. Vasohibin-1 affects hematogenous metastasis by regulating angiogenesis [14]. Overexpression of vasohibin-1 has been observed in numerous human malignancies, influencing various cellular functions, including cell proliferation, migration, and angiogenesis in both endothelial and cancer cells [14].

Given the complex nature of OSCC and its metastatic processes, it is essential to consider multiple biomarkers for a comprehensive understanding of disease progression and metastatic potential. The combination of sTIM-3, TGF- β 1, and vasohibin-1 was chosen due to their distinct roles in tumor biology. sTIM-3's immunosuppressive effects are critical for tumor

evasion and progression, TGF- β 1 reflects dynamic changes in the tumor microenvironment with its dual role in tumor suppression and promotion, and vasohibin-1 provides insight into vascular changes related to tumor spread. Combining these biomarkers may offer more reliable and multifaceted information for predicting lymph node metastasis in OSCC. Therefore, the present study aims to analyze the relationship between sTIM-3, TGF- β 1, and vasohibin-1 in OSCC metastasis by detecting their serum levels in OSCC patients, and to explore the efficacy of this combination for predicting OSCC metastasis. The goal is to provide a new combination of serological markers for risk assessment in OSCC metastasis and to offer a theoretical basis for clinical prediction.

Materials and methods

Sample size calculation

The sample size calculation was based on the requirement of at least ten events (cases of lymph node metastasis cases) per predictor variable, i.e., ten events per predictor variable [15]. Based on previous studies, the incidence of lymph node metastasis is estimated to be between 25% and 36% [16-18]. The total sample size required can be calculated using the following formula: Sample size=number of predictor variables $\times$ 10/incidence of events.

In this study, five predictive variables were planned for inclusion. Therefore, the sample size for the training set was calculated to be approximately 139 cases. Based on the 7:3 ratio of the training to validation sets, the number of samples in the validation set was approximately 60. Thus, a total of at least 199 cases needed to be included.

Study participants

This single-center cohort study included OSCC patients treated at Shanxi Provincial Cancer Hospital between January 2022 and December 2024. Postoperative pathological examination of lymph node metastasis revealed that 73 patients developed metastasis. The patient cohort was randomly divided into a training set (n=154) and a validation set (n=66). In the training set, there were 101 cases without lymph node metastasis and 53 cases with lymph node metastasis; in the validation set, there

sTIM-3, TGF- β 1, Vasohibin-1 in OSCC lymph node metastasis

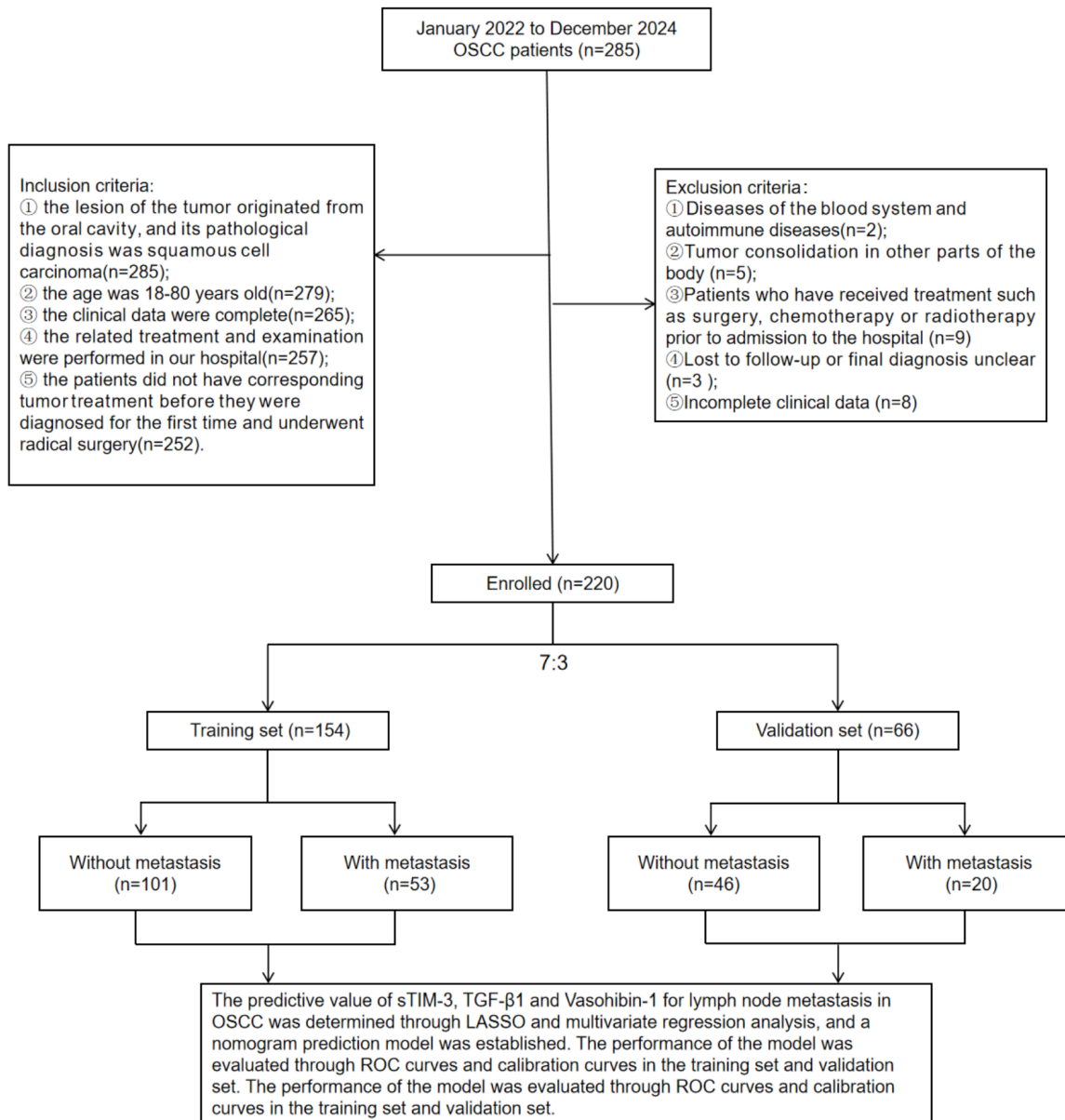


Figure 1. Study flow chart. OSCC: oral squamous cell carcinoma.

were 46 cases without lymph node metastasis and 20 cases with lymph node metastasis (**Figure 1**).

The study was approved by the Ethics Committee of Shanxi Provincial Cancer Hospital (Ethics Approval Number: KY2025166) and adhered to the ethical norms of the Declaration of Helsinki. As its retrospective nature, written patient consent was waived.

OSCC diagnostic criteria [19]: Microscopic pathological examination by cutting or excising diseased tissue remains the “gold standard”

for diagnosing OSCC, showing typical features such as abnormal squamous epithelial proliferation, keratinized bead formation, cellular anisotropy (large, deeply stained nuclei, pathological nuclear division), and mesenchymal infiltration. The gold standard for diagnosing lymph node metastasis is pathological histological examination, which involves obtaining lymph node tissue through surgery or biopsy and performing pathological analysis to check for cancer cell infiltration. This method is the ultimate diagnostic basis, relying on the microscopic observation of cell morphology to confirm tumor cell metastasis in the lymph nodes.

Inclusion criteria: (1) The tumor originated in the oral cavity and was diagnosed as squamous cell carcinoma. (2) Age between 18 and 80 years. (3) Complete clinical data. (4) Treatment and examinations were performed at our hospital. (5) No prior tumor treatment before diagnosis and radical surgery.

Exclusion criteria: (1) Presence of hematologic or autoimmune diseases that could affect the inflammation index. (2) Presence of tumors in other parts of the body. (3) Undergoing surgery, chemotherapy, or radiotherapy before admission. (4) Presence of hematologic, rheumatic, or other autoimmune diseases, or other conditions that could impact the inflammation index. (5) With tumors other than OSCC or secondary oral malignant tumors.

Data collection

Patients' clinical information was obtained from the hospital's electronic medical records. Baseline data included gender, age, clinical stage, depth of infiltration, histological grading, presence of extraperitoneal lymph node invasion, presence of nerve invasion, tumor site (tongue, buccal cavity, gingiva, floor of the mouth, palate), tumor diameter, history of oral disease, history of chronic conditions (e.g., hypertension, diabetes, heart disease, liver disease, kidney disease), smoking history, and alcohol consumption history.

Routine blood test data included white blood cell count, red blood cell count, platelet count, neutrophil count, total protein, albumin, globulin, and blood glucose.

Serum tumor markers included sTIM-3, TGF- β 1, vasohibin-1, squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), and carcinoembryonic antigen (CEA).

All serum samples were collected from patients after their initial OSCC diagnosis and before radical surgical treatment. The samples were tested simultaneously with routine blood tests and serum tumor markers. After collection, the serum was centrifuged and separated immediately. The samples were then dispensed and stored in an ultra-low-temperature freezer at -80°C. All tests were performed within the same batch to reduce batch-to-batch variation.

Serum levels of sTIM-3, TGF- β 1, and vasohibin-1 were measured using electrochemiluminescence immunoassay (ECLIA). The procedure was as follows: the kit instructions were strictly followed. Serum samples were mixed with magnetic beads coated with specific antibodies, and streptavidin magnetic beads were added to capture the complex. After washing, electrochemiluminescent substrate (tripropylamine) was added. The luminescence intensity was measured using an electrochemiluminescence immunoassay analyzer, and the concentration of each marker was determined based on the standard curve. The data for SCC-Ag, CYFRA21-1, and CEA were obtained from the hospital's laboratory department.

To ensure data quality, a standardized data collection form was developed. The form clearly outlined the requirements and options for each item to ensure comprehensive data collection from all research subjects, adhering to a unified standard and preventing omissions or format inconsistencies. A double data entry and validation mechanism was established to ensure that the data in the electronic database matched the original records, thereby improving the accuracy and completeness of the collected data.

Statistical analysis

Data analysis was conducted using the R 4.2.2 statistical software package. For categorical, data presented as frequency counts, the differences between groups were tested using the chi-squared test or Fisher's exact test. For continuous data, if the data followed a normal distribution, they were expressed as mean $\pm$ standard deviation (mean $\pm$ SD), and an independent samples t-test was used for comparisons between groups. For non-normally distributed data, the median (interquartile range) was used, and the Mann-Whitney U-test was applied for group comparisons. Differences were considered statistically significant when the *P*-value was less than 0.05.

Variables were screened using the least absolute shrinkage and selection operator (LASSO) regression method, and models were constructed using both univariate and multivariate logistic regression analysis. Column-line graphs were also plotted. Receiver operating charac-

teristic (ROC) curves were generated for both the training and validation sets to assess prediction accuracy. Calibration curves were used to evaluate the agreement between predicted and actual results, and clinical decision curve analysis (DCA) was performed to assess the clinical validity of the model.

Results

Comparison of baseline characteristics between training and validation sets

The baseline data of patients in the training and the validation sets were compared and analyzed, including both categorical data and continuous variables. The results indicated that there were no significant differences between the two groups in terms of categorical variables (e.g., gender, age, clinical stage, depth of invasion, histological grade, extranodal lymph node invasion, neurological involvement, tumor site, tumor diameter, history of oral disease, history of chronic diseases, smoking history, and alcohol consumption) and continuous variables (e.g., white blood cell count, red blood cell count, platelet count, neutrophil count, total protein, albumin, globulin, blood glucose, SCC-Ag, CYFRA21-1, CEA, sTIM-3, TGF- β 1, Vasohibin-1) (all $P > 0.05$, **Table 1**). The baseline characteristics of both groups were balanced and comparable, ensuring the reliability of the subsequent model development and validation.

Comparison of baseline characteristics between patients with and without lymph node metastases

In the training set, 51.49% (52/101) of non-metastatic patients were in stage I-II, compared to 28.30% (15/53) of metastatic patients ($\chi^2=7.601$, $P=0.006$). In the validation set, 69.57% (32/46) of non-metastatic patients were in stage I-II, compared to 35.00% (7/20) of metastatic patients ($\chi^2=6.889$, $P=0.009$). Regarding tumor diameter, 57.43% (58/101) of non-metastatic patients in the training set had a tumor diameter < 3 cm, compared to 32.08% (17/53) of metastatic patients ($\chi^2=8.941$, $P=0.003$). In the validation set, 71.74% (33/46) of non-metastatic patients had a tumor diameter < 3 cm, compared to 20.00% (4/20) of metastatic patients ($\chi^2=15.149$, $P < 0.001$). However, no significant differences were found between

the two groups in terms of gender, age, depth of invasion, histological grade, extranodal lymph node invasion, neurological involvement, tumor site, history of oral diseases, chronic diseases, smoking history, and alcohol consumption (all $P > 0.05$, **Table 2**).

Comparison of blood routine data between patients with and without lymph node metastases

For white blood cell count, the training set showed $5.54 \pm 1.27 \times 10^9/L$ in non-metastatic patients, compared to $5.98 \pm 0.62 \times 10^9/L$ in metastatic patients ($t=2.375$, $P=0.019$). In the validation set, non-metastatic patients had $5.76 \pm 1.00 \times 10^9/L$, compared to $6.77 \pm 0.70 \times 10^9/L$ in metastatic patients ($t=2.067$, $P=0.043$). For red blood cell count, the training set had $4.26 \pm 0.36 \times 10^{12}/L$ in non-metastatic patients versus $4.39 \pm 0.33 \times 10^{12}/L$ in metastatic patients ($t=2.190$, $P=0.030$). In the validation set, non-metastatic patients had $4.24 \pm 0.33 \times 10^{12}/L$, compared to $4.43 \pm 0.36 \times 10^{12}/L$ in metastatic patients ($t=2.091$, $P=0.040$). However, no significant differences were found between the two groups in platelet count, neutrophil count, total protein, albumin, globulin, or blood glucose levels in either set (all $P > 0.05$, **Table 3**).

Comparison of serum tumor markers between patients with and without lymph node metastases

In the training set, patients with lymph node metastasis had significantly higher levels of SCC-Ag, CYFRA21-1, and CEA compared to non-metastatic patients (all $P < 0.001$, **Table 4** and **Figure 2**). Similarly, in the validation set, the metastatic group exhibited higher levels of these markers compared to the non-metastatic group (all $P < 0.001$, **Table 4**).

LASSO regression screening of prognostic factors for the occurrence of lymphatic metastases in training set

A total of 27 variables, including baseline data, blood routine data, and serum tumor markers, were included in the LASSO regression analysis for variable selection. The optimal parameter (lambda) for the LASSO regression was determined by cross-validation, with the value of lambda that produced the smallest mean-

Table 1. Comparison baseline data between training and validation sets

Characteristic	Training set (N=154)	Validation set (N=66)	t/χ ²	P
Gender, n (%)			0.979	0.323
Female	59 (38.31%)	30 (45.45%)		
Male	95 (61.69%)	36 (54.54%)		
Age, n (%)			0.008	0.930
<60 years	76 (49.35%)	33 (50.00%)		
≥60 years	78 (50.65%)	33 (50.00%)		
Clinical stage, n (%)			4.494	0.034
I-II	67 (43.51%)	39 (59.09%)		
III-IV	87 (56.49%)	27 (40.91%)		
Depth of invasion, n (%)			0.153	0.926
<5 mm	37 (24.03%)	15 (22.73%)		
5-10 mm	61 (39.61%)	28 (42.42%)		
>10 mm	56 (36.36%)	23 (34.85%)		
Histological grade, n (%)			0.352	0.839
I	48 (31.17%)	18 (27.27%)		
II	74 (48.05%)	33 (50.00%)		
III	32 (20.78%)	15 (22.73%)		
Extranodal invasion of the lymph node capsule, n (%)			2.538	0.111
Yes	88 (57.14%)	30 (45.45%)		
No	66 (42.86%)	36 (54.55%)		
Neurological involvement, n (%)			0.171	0.680
Yes	84 (54.55%)	34 (51.52%)		
No	70 (45.45%)	32 (48.48%)		
Tumor site, n (%)			1.604	0.808
tongue	42 (27.27%)	15 (22.73%)		
Buccal	27 (17.53%)	16 (24.24%)		
Gingiva	28 (18.18%)	11 (16.67%)		
Floor of mouth	31 (20.13%)	14 (21.21%)		
Palate	26 (16.88%)	10 (15.15%)		
Tumor diameter, n (%)			1.001	0.317
<3 mm	75 (48.70%)	37 (56.06%)		
≥3 mm	79 (51.30%)	29 (43.94%)		
History of oral disease, n (%)			0.458	0.499
Yes	80 (51.95%)	31 (46.97%)		
No	74 (48.05%)	35 (53.03%)		
History of chronic diseases, n (%)			0.571	0.450
Yes	92 (59.74%)	43 (65.15%)		
No	62 (40.26%)	23 (34.85%)		
Smoking history, n (%)			0.842	0.359
Yes	101 (65.58%)	39 (59.09%)		
No	53 (34.42%)	27 (40.91%)		
Alcohol history, n (%)			0.906	0.341
Yes	85 (55.19%)	41 (62.12%)		
No	69 (44.81%)	25 (37.88%)		
White blood cell count, (× 10 ⁹ /L)	5.74 ± 1.58	5.63 ± 1.90	0.396	0.693
Red blood cell count, (× 10 ¹² /L),	4.23 ± 0.55	4.09 ± 0.50	1.894	0.060
Platelet count, (× 10 ⁹ /L)	289.91 ± 89.25	294.83 ± 95.04	-0.358	0.721
Neutrophil count, (× 10 ⁹ /L)	3.54 ± 0.62	3.50 ± 0.63	0.453	0.652
Total protein, (g/L)	68.82 ± 19.66	71.52 ± 18.15	-0.985	0.326

sTIM-3, TGF-β1, Vasohibin-1 in OSCC lymph node metastasis

Albumin, (g/L)	41.66 ± 2.02	41.65 ± 1.99	-0.009	0.993
Globulin, (g/L)	25.84 ± 2.04	25.74 ± 2.06	0.342	0.733
Blood glucose, (mmol/L)	8.17 ± 0.69	8.17 ± 0.82	-0.031	0.975
SCC-Ag, (μg/L)	5.86 ± 1.69	5.86 ± 2.14	0.009	0.993
CYFRA21-1, (μg/L)	4.44 ± 1.16	4.26 ± 1.07	1.127	0.262
CEA, (μg/L)	4.68 ± 1.47	4.68 ± 1.34	0.012	0.990
sTIM-3, (ng/L)	6,531.75 ± 1,535.71	6,164.93 ± 1,628.26	1.557	0.122
TGF-β1, (μg/L)	22.38 ± 4.70	23.10 ± 4.63	-1.048	0.297
Vasohibin-1, (ng/L)	557.96 ± 95.03	570.56 ± 96.97	-0.888	0.376

Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3; TGF-β1, Transforming Growth Factor-β1.

square error selected as the optimal model value ($\lambda=0.0112772162210942$). Based on the coefficients obtained from the LASSO regression analysis, the final selected variables were clinical stage, tumor diameter, SCC-Ag, CEA, sTIM-3, TGF-β1, and Vasohibin-1 (**Figure 3**).

Univariate and multivariate logistic regression analyses in training set

The co-linearity test results indicated no collinearity among clinical stage, tumor diameter, SCC-Ag, CEA, sTIM-3, TGF-β1, and Vasohibin-1 (**Table 5**). The optimal cutoff values for predicting lymph node metastasis using SCC-Ag, CEA, sTIM-3, TGF-β1, and Vasohibin-1 were determined through ROC curve analysis (**Table 6**; **Figure 4**). The cutoff values were established using the Youden index (sensitivity specificity-1). The optimal cutoff values were as follows: SCC-Ag at 6.58 μg/L, CEA at 5.11 μg/L, sTIM-3 at 7596.60 ng/L, TGF-β1 at 23.25 μg/L, and Vasohibin-1 at 611.40 ng/L.

In the training set, the univariate analysis revealed that clinical stage (OR=2.688, 95% CI=1.317~5.489, P=0.007), tumor diameter (OR=2.856, 95% CI=1.420~5.745, P=0.003), SCC-Ag (OR=3.641, 95% CI=2.359~5.620, P=0.001), CEA (OR=8.313, 95% CI=4.181~16.528, P=0.001), sTIM-3 (OR=1.001, 95% CI=1.001~1.001, P=0.006), TGF-β1 (OR=1.272, 95% CI=1.157~1.399, P=0.001), and Vasohibin-1 (OR=1.007, 95% CI=1.003~1.011, P=0.001) were significantly associated with lymph node metastasis (**Table 7**).

Multivariate analysis further identified clinical stage (OR=3.221, 95% CI=1.244~8.341, P=0.016), tumor diameter (OR=2.692, 95% CI=1.527~4.350, P=0.081), SCC-Ag (OR=5.298,

95% CI=2.269~12.372, P=0.001), and TGF-β1 (OR=2.633, 95% CI=1.209~5.376, P=0.015) as independent predictors of lymph node metastasis (**Table 7**).

Construction, performance evaluation and calibration of nomogram model

To further assess the clinical utility of the model, column-line plots were constructed based on multifactorial logistic regression (**Figure 5**). In the training set, a predictive model was developed using the five independent predictors identified through multivariate regression analysis: $\log(\text{probability}) = 24.967 + 1.170(\text{clinical stage III-IV}) + 1.667(\text{SCC-Ag} \geq 6.58) + 0.001(\text{sTIM-3} \geq 7596.60) + 0.968(\text{TGF-}\beta 1 \geq 23.25) + 0.030(\text{Vasohibin-1} \geq 611.40)$.

Compared to clinical stage I-II patients, stage III-IV patients had $\beta=1.170$, indicating a significantly increased risk of lymph node metastasis (OR=3.221, 95% CI: 1.244~8.341, P=0.016). For SCC-Ag ≥ 6.58 μg/L, patients had $\beta=1.667$, indicating a significantly increased risk of metastasis (OR=5.298, 95% CI: 2.269~12.372, P=0.001). Although sTIM-3 exhibited a smaller coefficient ($\beta=0.001$), it retained predictive significance beyond the threshold (≥ 7596.60 ng/L). For every 1000 ng/L increase in sTIM-3, the risk of metastasis increased by approximately 2.718-fold (OR=1.001, 95% CI: 1.000~1.002, P=0.045). For TGF-β1 ≥ 23.25 μg/L, $\beta=0.968$, indicating increased metastasis risk (OR=2.633, 95% CI: 1.209~5.376, P=0.015). For Vasohibin-1 ≥ 611.40 ng/L, $\beta=0.030$, indicating a mild increase in metastasis risk (OR=1.030, 95% CI: 1.003~1.058, P=0.029). For every 100 ng/L increase in Vasohibin-1, the odds ratio for metastasis increased by approximately 1.030.

sTIM-3, TGF-β1, Vasohibin-1 in OSCC lymph node metastasis

Table 2. Comparison of Baseline data between patients with and without lymph node metastases

Characteristic	Training set (N=154)				Validation set (N=66)			
	Without metastasis (N=101)	With metastasis (N=53)	χ^2	P	Without metastasis (N=46)	With metastasis (N=20)	χ^2	P
Gender, n (%)			2.256	0.133			2.449	0.118
Male	58 (57.43%)	37 (69.81%)			28 (60.87%)	8 (40.00%)		
Female	43 (42.57%)	16 (30.19%)			18 (39.13%)	12 (60.00%)		
Age, n (%)			0.003	0.958			0.287	0.592
<60 years	50 (49.50%)	26 (49.06%)			24 (52.17%)	9 (45.00%)		
≥60 years	51 (50.50%)	27 (50.94%)			22 (47.83%)	11 (55.00%)		
Clinical stage, n (%)			7.601	0.006			6.889	0.009
I-II	52 (51.49%)	15 (28.30%)			32 (69.57%)	7 (35.00%)		
III-IV	49 (48.51%)	38 (71.70%)			14 (30.43%)	13 (65.00%)		
Depth of invasion, n (%)			3.618	0.164			4.982	0.083
<5 mm	28 (27.72%)	9 (16.98%)			9 (19.57%)	6 (30.00%)		
5-10 mm	35 (34.65%)	26 (49.06%)			17 (36.96%)	11 (55.00%)		
>10 mm	38 (37.62%)	18 (33.96%)			20 (43.48%)	3 (15.00%)		
Histological grade, n (%)			0.034	0.983			0.976	0.614
I	31 (30.69%)	17 (32.08%)			12 (26.09%)	6 (30.00%)		
II	49 (48.51%)	25 (47.17%)			22 (47.83%)	11 (55.00%)		
III	21 (20.79%)	11 (20.75%)			12 (26.09%)	3 (15.00%)		
Extranodal invasion of the lymph node capsule, n (%)			3.836	0.050			2.449	0.118
Yes	52 (51.49%)	36 (67.92%)			18 (39.13%)	12 (60.00%)		
No	49 (48.51%)	17 (32.08%)			28 (60.87%)	8 (40.00%)		
Neurological involvement, n (%)			1.109	0.292			2.089	0.148
Yes	52 (51.49%)	32 (60.38%)			21 (45.65%)	13 (65.00%)		
No	49 (48.51%)	21 (39.62%)			25 (54.35%)	7 (35.00%)		
Tumor site, n (%)			1.686	0.793			3.334	0.503
tongue	26 (25.74%)	16 (30.19%)			10 (21.74%)	5 (25.00%)		
Buccal	17 (16.83%)	10 (18.87%)			13 (28.26%)	3 (15.00%)		
Gingiva	21 (20.79%)	7 (13.21%)			8 (17.39%)	3 (15.00%)		
Floor of mouth	21 (20.79%)	10 (18.87%)			8 (17.39%)	6 (30.00%)		
Palate	16 (15.84%)	10 (18.87%)			7 (15.22%)	3 (15.00%)		
Tumor diameter, n (%)			8.941	0.003			15.149	<0.001
<3 mm	58 (57.43%)	17 (32.08%)			33 (71.74%)	4 (20.00%)		
≥3 mm	43 (42.57%)	36 (67.92%)			13 (28.26%)	16 (80.00%)		
History of oral disease, n (%)			0.025	0.874			0.864	0.353
Yes	52 (51.49%)	28 (52.83%)			37 (80.43%)	14 (70.00%)		
No	49 (48.51%)	25 (47.17%)			9 (19.57%)	6 (30.00%)		

sTIM-3, TGF-β1, Vasohibin-1 in OSCC lymph node metastasis

History of chronic diseases, n (%)			0.014	0.907			0.000	0.986
Yes	60 (59.41%)	32 (60.38%)			30 (65.22%)	13 (65.00%)		
No	41 (40.59%)	21 (39.62%)			16 (34.78%)	7 (35.00%)		
Smoking history, n (%)			0.007	0.932			0.981	0.322
Yes	66 (65.35%)	35 (66.04%)			29 (63.04%)	10 (50.00%)		
No	35 (34.65%)	18 (33.96%)			17 (36.96%)	10 (50.00%)		
Alcohol history, n (%)			0.065	0.799			0.618	0.432
Yes	46 (45.54%)	23 (43.40%)			16 (34.78%)	9 (45.00%)		
No	55 (54.46%)	30 (56.60%)			30 (65.22%)	11 (55.00%)		

Table 3. Comparison of blood routine data between patients with and without lymph node metastases

Characteristic	Training set (N=154)				Validation set (N=66)			
	Without metastasis (N=101)	With metastasis (N=53)	t	P	Without metastasis (N=46)	With metastasis (N=20)	t	P
White blood cell count, ($\times 10^9/L$)	5.54 $\pm$ 1.27	5.98 $\pm$ 0.62	2.375	0.019	5.76 $\pm$ 1.00	6.77 $\pm$ 0.70	2.067	0.043
Red blood cell count, ($\times 10^{12}/L$)	4.26 $\pm$ 0.36	4.39 $\pm$ 0.33	2.190	0.030	4.24 $\pm$ 0.33	4.43 $\pm$ 0.36	2.091	0.040
Platelet count, ($\times 10^9/L$)	298.18 $\pm$ 99.96	286.02 $\pm$ 50.20	1.005	0.317	262.11 $\pm$ 96.35	288.88 $\pm$ 59.39	-1.377	0.174
Neutrophil count, ($\times 10^9/L$)	3.51 $\pm$ 0.57	3.61 $\pm$ 0.71	-0.942	0.349	3.40 $\pm$ 0.60	3.74 $\pm$ 0.66	-1.988	0.055
Total protein, (g/L)	69.48 $\pm$ 16.61	67.56 $\pm$ 24.59	0.508	0.613	71.85 $\pm$ 16.95	70.74 $\pm$ 21.11	0.208	0.837
Albumin, (g/L)	41.45 $\pm$ 2.04	42.06 $\pm$ 1.94	-1.843	0.068	41.79 $\pm$ 1.95	41.36 $\pm$ 2.09	0.797	0.431
Globulin, (g/L)	25.61 $\pm$ 1.98	26.28 $\pm$ 2.11	-1.928	0.057	25.69 $\pm$ 2.23	25.85 $\pm$ 1.65	-0.317	0.753
Blood glucose, (mmol/L)	8.17 $\pm$ 0.74	8.16 $\pm$ 0.58	0.130	0.897	8.32 $\pm$ 0.81	8.45 $\pm$ 0.78	0.606	0.546

Table 4. Comparison of serum tumor markers between patients with and without lymph node metastases

Characteristic	Training set (N=154)				Validation set (N=66)			
	Without metastasis (N=101)	With metastasis (N=53)	t	P	Without metastasis (N=46)	With metastasis (N=20)	t	P
SCC-Ag, ($\mu g/L$)	5.10 $\pm$ 1.00	7.30 $\pm$ 1.79	-8.283	<0.001	4.96 $\pm$ 0.87	7.92 $\pm$ 2.73	-4.753	<0.001
CYFRA21-1, ($\mu g/L$)	3.96 $\pm$ 0.81	5.37 $\pm$ 1.18	-7.803	<0.001	3.84 $\pm$ 0.78	5.22 $\pm$ 1.03	-5.368	<0.001
CEA, ($\mu g/L$)	3.89 $\pm$ 0.87	6.18 $\pm$ 1.19	-12.393	<0.001	4.09 $\pm$ 0.97	6.05 $\pm$ 1.06	-7.062	<0.001
sTIM-3, (ng/L)	6,282.60 $\pm$ 1,538.58	7,006.55 $\pm$ 1,427.04	-2.911	0.004	5,722.70 $\pm$ 1,480.63	7,182.06 $\pm$ 1,521.63	-3.610	<0.001
TGF-β1, ($\mu g/L$)	20.87 $\pm$ 4.20	25.25 $\pm$ 4.28	-6.066	<0.001	21.62 $\pm$ 3.87	26.49 $\pm$ 4.52	-4.199	<0.001
Vasohibin-1, (ng/L)	536.78 $\pm$ 85.23	598.32 $\pm$ 100.35	-3.803	<0.001	557.47 $\pm$ 89.01	605.67 $\pm$ 91.71	-2.004	0.049

Note: The serum tumor markers follow a normal distribution. SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain - 3; TGF-β1, Transforming Growth Factor-β1.

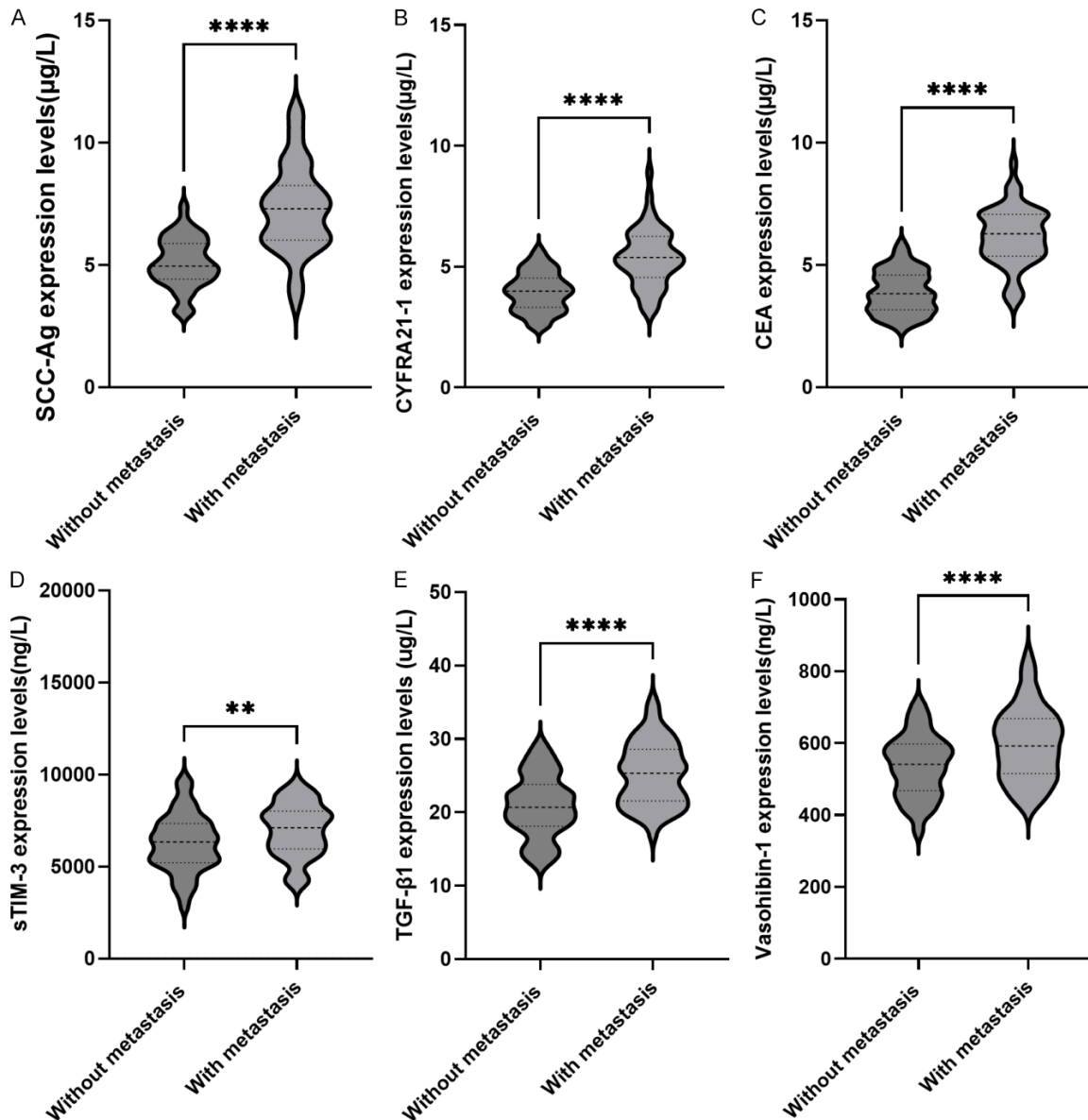


Figure 2. Comparison of serum tumor marker levels between OSCC patients with and without lymphatic metastases. ** $P < 0.01$. **** $P < 0.001$. OSCC: oral squamous cell carcinoma. A. Comparison of SCC-Ag expression levels between patients with and without lymph node metastasis in the training set. B. Comparison of CYFRA21-1 expression levels between patients with and without lymph node metastasis in the training set. C. Comparison of CEA expression levels between patients with and without lymph node metastasis in the training set. D. Comparison of sTIM-3 expression levels between patients with and without lymph node metastasis in the training set. E. Comparison of TGF- β 1 expression levels between patients with and without lymph node metastasis in the training set. F. Comparison of Vasohibin-1 expression levels between patients with and without lymph node metastasis in the training set. Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3, TGF- β 1, Transforming Growth Factor- β 1.

In the training set, the area under the curve (AUC) of the ROC curve was 0.868, indicating high prediction accuracy as the value exceeded 0.7. Similarly, in the validation set, the ROC analysis showed an AUC of 0.863, confirming the model's robust predictive performance (Figure 6).

Calibration curves were used to assess the consistency between the predicted probabilities from the column-line plots and the actual observed frequencies. The calibration curves (Figure 7) demonstrate the model's performance across different probability thresholds. Ideally, all points should fall along a 45-degree

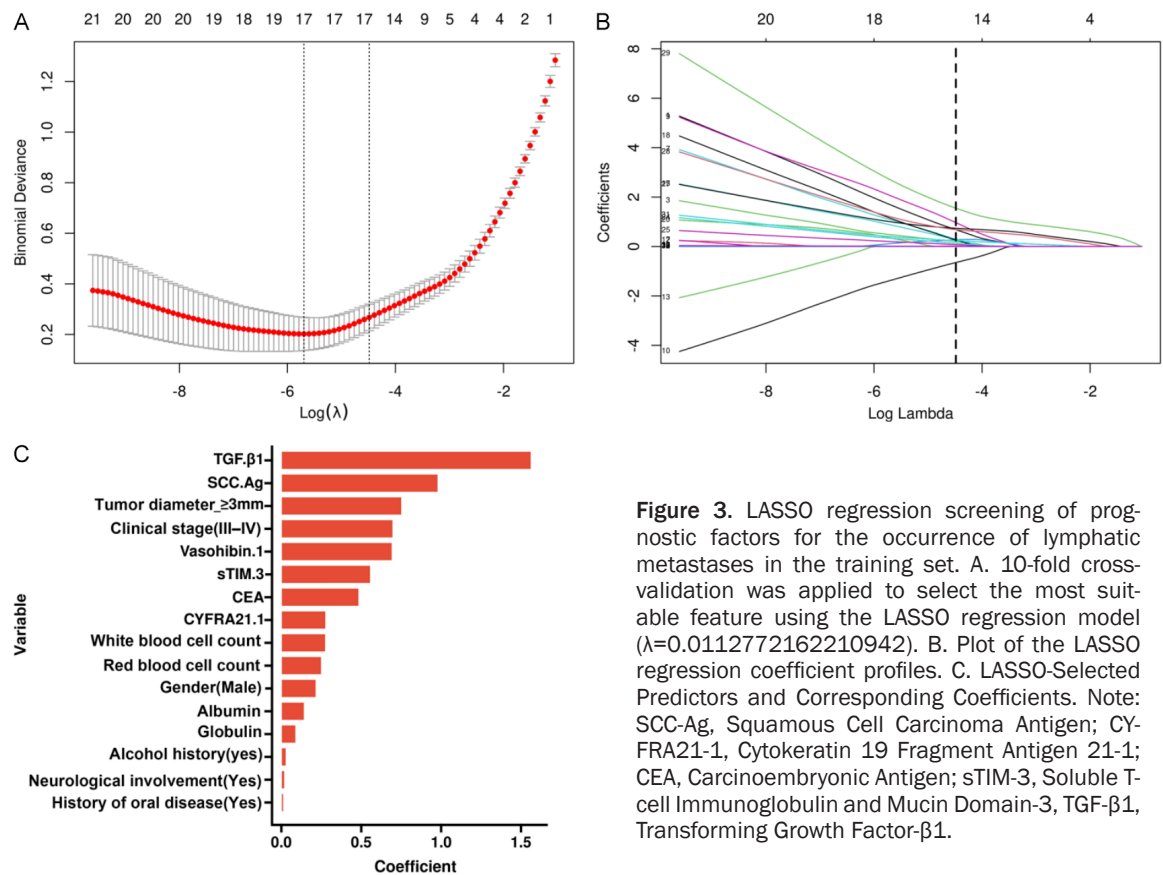


Figure 3. LASSO regression screening of prognostic factors for the occurrence of lymphatic metastases in the training set. A. 10-fold cross-validation was applied to select the most suitable feature using the LASSO regression model ($\lambda=0.0112772162210942$). B. Plot of the LASSO regression coefficient profiles. C. LASSO-Selected Predictors and Corresponding Coefficients. Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CYFRA21-1, Cytokeratin 19 Fragment Antigen 21-1; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3, TGF-β1, Transforming Growth Factor-β1.

Table 5. Co-linearity test (Training set)

Variables	Tolerance	VIF
Clinical stage	0.957	1.045
Tumor diameter	0.960	1.042
SCC-Ag	0.752	1.331
CEA	0.675	1.482
sTIM-3	0.950	1.053
TGF-β1	0.884	1.131
Vasohibin-1	0.950	1.053

Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3; TGF-β1, Transforming Growth Factor-β1.

diagonal line, indicating perfect calibration. In the training set, the model's predictions were closer to the actual occurrences, and the calibration curve was closer to the ideal line. In the validation set, the calibration curve remained relatively close to the ideal curve, demonstrating a high degree of agreement between predicted and actual values. The p -values for goodness of fit were 0.865 for the training set and 0.872 for the validation set, indicating

strong agreement between predicted and actual values.

Figure 8 presents the decision curve analysis, which plots threshold probability (x-axis) against net benefit (y-axis). The red line represents the net benefit of the predictive model. Clinical decision curve analysis showed that the net benefit values for both the training and validation sets were essentially the same. Both models provided significant net benefits for clinical decision-making at most thresholds. The analysis demonstrated a significant clinical benefit, with maximum benefit rates of 39.41% for the training set and 37.80% for the validation set.

Discussion

This study developed a nomogram-based predictive model for lymph node metastasis in OSCC via LASSO and logistic regression analyses, which exhibited promising utility in clinical risk assessment. Clinical stage, SCC-Ag, sTIM-3, TGF-β1, and Vasohibin-1 were identified as independent risk factors for OSCC lymph node metastasis. The model demonstrated excellent

Table 6. The predictive value of variables for lymph node metastasis in OSCC patients

Variables	AUC	95% CI	Cutoff Value	Sensitivity	Specificity	Youden index
Clinical stage	0.616	0.538~0.694	/	0.717	0.515	0.232
Tumor diameter	0.627	0.547~0.707	/	0.679	0.574	0.255
SCC-Ag	0.864	0.797~0.932	6.58 (μg/L)	0.642	0.951	0.592
CEA	0.936	0.893~0.978	5.11 (μg/L)	0.868	0.931	0.799
sTIM-3	0.645	0.553~0.736	7596.60 (ng/L)	0.415	0.832	0.247
TGF-β1	0.758	0.681~0.835	23.25 (μg/L)	0.623	0.743	0.365
Vasohibin-1	0.668	0.577~0.760	611.40 (ng/L)	0.472	0.832	0.303

Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3; TGF-β1, Transforming Growth Factor-β1. AUC, Area Under the Curve.

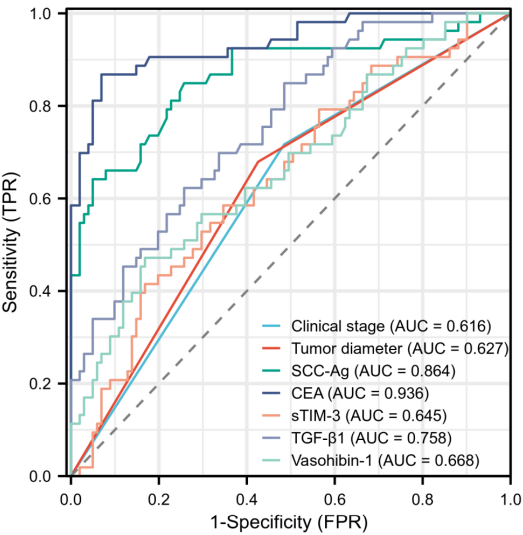


Figure 4. The ROC curve for the predictive value of variables for lymph node metastasis in OSCC patients. OSCC: oral squamous cell carcinoma. Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3, TGF-β1, Transforming Growth Factor-β1.

discriminative power and calibration accuracy in both training and validation cohorts, confirming its clinical value for predicting OSCC lymph node metastasis. These findings clarify key high-risk factors, providing a scientific basis for individualized risk stratification and precise intervention to improve patient prognosis.

This study confirmed associations between clinical stage and tumor diameter with OSCC lymph node metastasis. Clinical stage, a well-established indicator of overall tumor progression [20], correlates with lymph node metastasis risk: advanced stages typically involve larger tumor volume and broader invasion, increasing lymphatic vessel infiltration [21]. Consistent

with prior research [22], OSCC lymph node metastasis rates rise with advancing clinical stage. Tumor diameter ≥ 3 mm is also relevant, as it reflects progressive tumor cell proliferation and invasiveness-larger tumors cause greater compression and infiltration of surrounding tissues, disrupting local lymphatic networks and facilitating lymphatic invasion [23]. Routine blood analysis revealed significantly higher leukocyte and erythrocyte counts in the metastatic group, consistent with previous reports [24-26]. Elevated leukocytes may stem from tumor-induced systemic inflammation, as tumor cells stimulate the immune system to trigger inflammatory responses.

In terms of serum tumor markers, SCC-Ag, CYFRA21-1, CEA, sTIM-3, TGF-β1, and Vasohibin-1 were significantly elevated in the metastatic group compared to the non-metastatic group in both cohorts consistent with prior findings. SCC-Ag, a squamous cell carcinoma-specific marker, is closely linked to OSCC invasion and metastasis [27], while CYFRA21-1 and CEA-broad-spectrum tumor markers [28] - were also upregulated in metastatic OSCC, suggesting potential roles in regulating metastasis. Notably, the roles of sTIM-3, TGF-β1, and Vasohibin-1 in OSCC metastasis remain incompletely understood; this study provides novel evidence for their involvement by characterizing their differential expression.

LASSO regression screened seven variables (clinical stage, tumor diameter, SCC-Ag, CEA, sTIM-3, TGF-β1, Vasohibin-1), and multivariate logistic regression identified clinical stage, SCC-Ag, sTIM-3, TGF-β1, and Vasohibin-1 as independent predictors. Their respective roles in OSCC lymph node metastasis are elaborated as follows.

Table 7. Univariate factor and multivariate factor Logistic regression analysis of training set

Variables	Univariate					Multivariate				
	β	S.E	Z	P	OR (95% CI)	β	S.E	Z	P	OR (95% CI)
Clinical stage										
I-II					1.000 (Reference)					1.000 (Reference)
III-IV	0.989	0.364	2.716	0.007	2.688 (1.317~5.489)	1.170	0.486	2.407	0.016	3.221 (1.244, 8.341)
Tumor diameter (mm)										
<3					1.000 (Reference)					1.000 (Reference)
≥3	1.050	0.357	2.944	0.003	2.856 (1.420~5.745)	5.152	2.956	1.743	0.081	2.692 (1.527, 4.350)
SCC-Ag (μg/L)										
<6.58					1.000 (Reference)					1.000 (Reference)
≥6.58	1.292	0.221	5.835	0.001	3.641 (2.359~5.620)	1.667	0.433	3.873	0.001	5.298 (2.269~12.372)
CEA (μg/L)										
<5.11					1.000 (Reference)					1.000 (Reference)
≥5.11	2.118	0.351	6.040	0.001	8.313 (4.181~16.528)	2.566	1.445	1.776	0.056	1.247 (0.169~5.520)
sTIM-3 (ng/L)										
<7596.60					1.000 (Reference)					1.000 (Reference)
≥7596.60	0.001	0.000	2.727	0.006	1.001 (1.001~1.001)	0.001	0.000	2.362	0.045	1.001 (1.000~1.002)
TGF-β1 (μg/L)										
<23.25					1.000 (Reference)					1.000 (Reference)
≥23.25	0.241	0.048	4.984	0.001	1.272 (1.157~1.399)	0.968	0.397	2.438	0.015	2.633 (1.209~5.376)
Vasohibin-1 (ng/L)										
<611.40					1.000 (Reference)					1.000 (Reference)
≥611.40	0.007	0.002	3.642	0.001	1.007 (1.003~1.011)	0.030	0.014	2.143	0.029	1.030 (1.003~1.058)

Note: Cut-off values for continuous indicators were determined by ROC curve analysis with maximum Youden's Index. OR: Odds Ratio, CI: Confidence Interval; SCC-Ag, Squamous Cell Carcinoma Antigen; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3; TGF-β1, Transforming Growth Factor-β1.

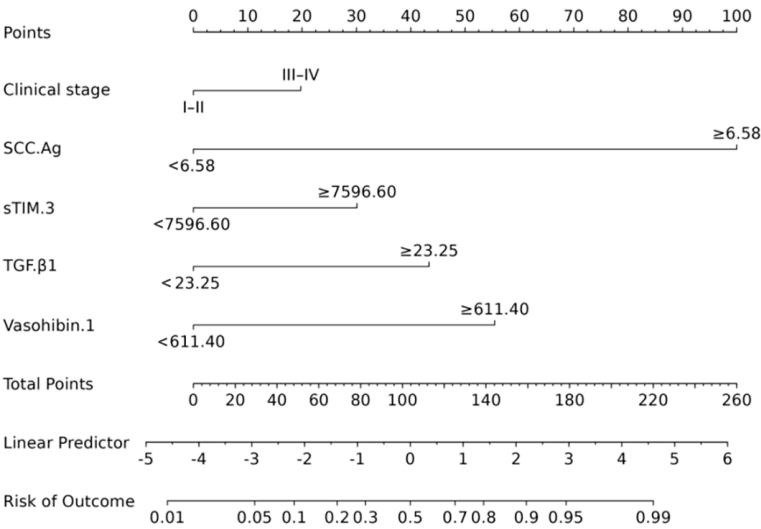


Figure 5. Nomogram prediction model based on clinical stage, SCC-Ag, sTIM-3, TGF-β1 and Vasohibin-1. Note: SCC-Ag, Squamous Cell Carcinoma Antigen; CEA, Carcinoembryonic Antigen; sTIM-3, Soluble T-cell Immunoglobulin and Mucin Domain-3; TGF-β1, Transforming Growth Factor-β1.

As a squamous cell carcinoma-specific marker, SCC-Ag exhibits high relevance in predicting lymph node metastasis. Elevated SCC-Ag levels often indicate active proliferation and enhanced invasiveness of tumor cells. Secreted into the bloodstream by tumor cells, its concentration

directly reflects tumor burden and metastatic potential [27], providing a reliable basis for clinical assessment of lymph node metastasis risk.

sTIM-3 contributes to metastasis primarily by mediating immune escape. It inhibits immune surveillance function through blocking the binding of TIM-3 to its ligands [29], creating favorable conditions for OSCC cells to evade immune attack during lymph node invasion. This molecule suppresses T cell immune activity via multiple pathways, a mechanism consistent with its role in other malignancies-elevated serum sTIM-3 levels are commonly associated with advanced tumor stages,

increased tumor burden, and shortened patient survival [9, 30]. For instance, in non-small cell lung cancer, sTIM-3 shows higher diagnostic accuracy compared to other soluble immune checkpoint molecules like sLAG-3 and SPD-L1 [31, 32]; in clear cell renal cell carcinoma, its

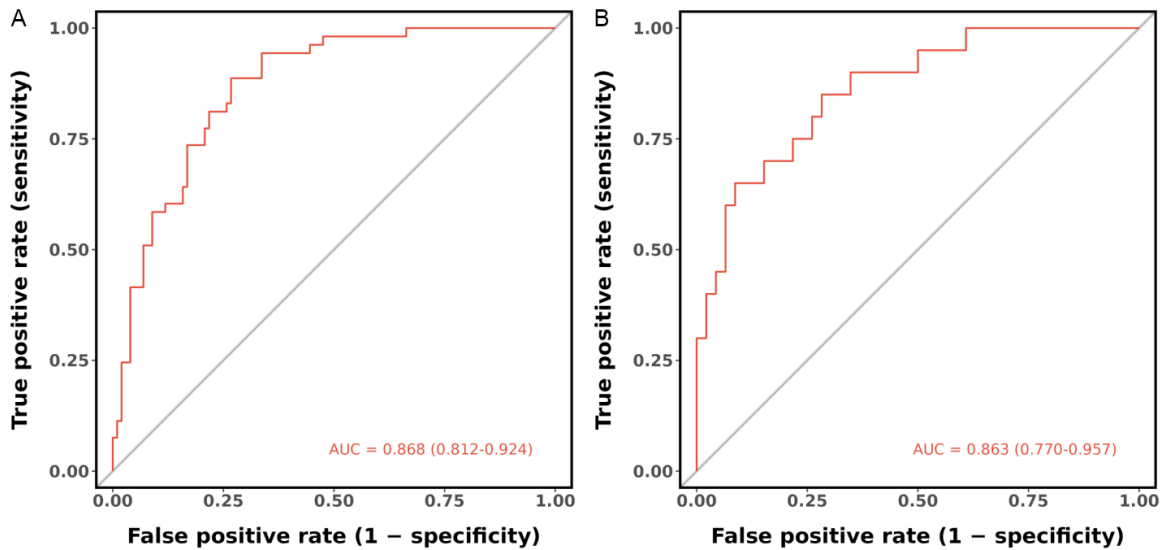


Figure 6. ROC curves for predictive modeling of occurrence of lymphatic metastases. A. Training set ROC curve. B. Validation set ROC curve.

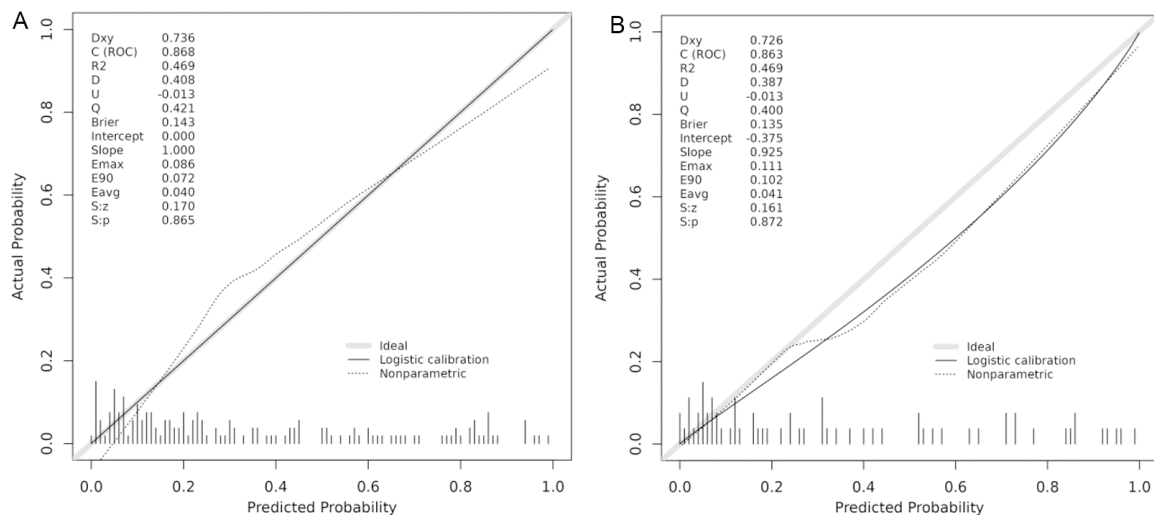


Figure 7. Calibration curves for the prediction of lymphatic metastases by a column-line graph model. A. Training set calibration curve. B. Validation set calibration curve.

elevated expression correlates with advanced stage III disease and reduced survival, serving as a potential biomarker for disease severity and prognosis [33].

TGF- β 1 drives lymph node metastasis mainly through inducing epithelial-mesenchymal transition. This biological process transforms polarized, tightly adherent epithelial cells into mesenchymal-like cells with enhanced migration and invasion capabilities, enabling tumor cells to infiltrate surrounding tissues and reach regional lymph nodes [34]. Additionally, TGF- β 1

modulates the tumor microenvironment by promoting fibrosis and immunosuppression, further facilitating tumor cell dissemination—an effect that aligns with its association with poor prognosis in OSCC [35]. Consistent with previous research [36], this study observed increased TGF- β 1 expression in OSCC patients with lymph node metastasis, which may be attributed to its multifaceted pro-metastatic effects, including promoting tumor cell detachment, proliferation, angiogenesis, matrix metalloproteinase induction, and enhancing cell invasiveness and motility [37].

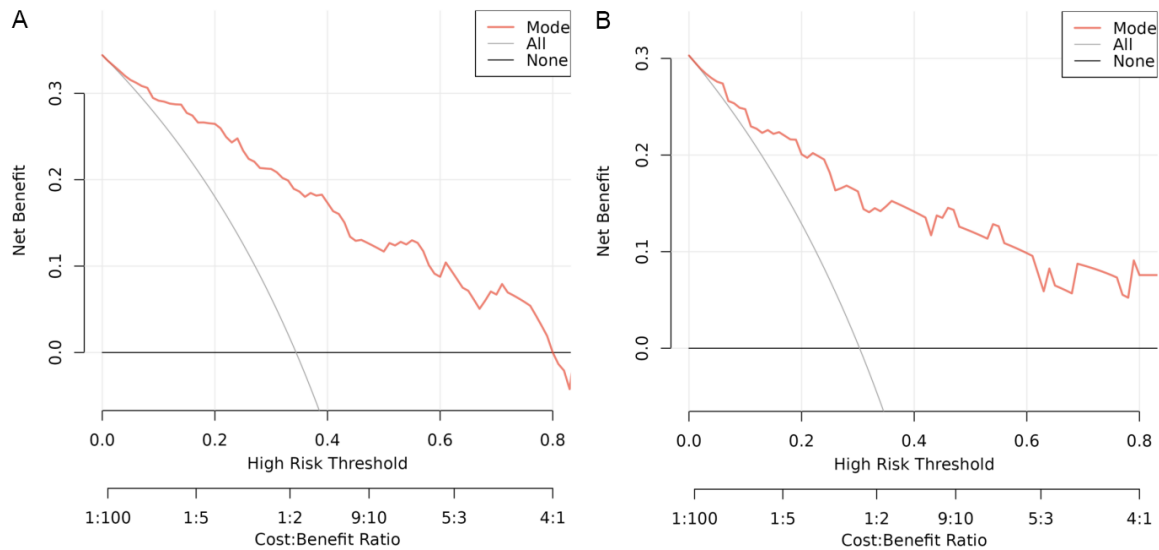


Figure 8. Clinical decision-making (DCA) curves for predicting lymphatic metastases by column-line diagram modeling. A. Training set DCA curve. B. Validation set DCA curve.

Vasohibin-1, a negative regulator of angiogenesis, participates in metastasis by disrupting vascular homeostasis. Its abnormal expression disturbs the balanced regulation of tumor angiogenesis, leading to the formation of disordered vascular networks. These structurally abnormal vessels provide physical channels for OSCC cells to enter the circulatory system and colonize lymph nodes [38, 39]. Similar to its role in ovarian and breast cancers [40, 41], Vasohibin-1 dysregulation in OSCC affects not only angiogenesis but also lymphangiogenesis, collectively promoting the metastatic spread of cancer cells to lymph nodes. Its status as a poor prognostic factor in various malignancies further underscores its critical role in driving metastatic processes [42].

Notably, TGF- β 1 levels were positively correlated with sTIM-3 levels in the metastatic group, suggesting a potential synergistic effect-TGF- β 1 may upregulate sTIM-3 to enhance immunosuppression and invasiveness. Elevated Vasohibin-1 may further interact with sTIM-3-mediated immune escape and TGF- β 1-driven EMT, collectively promoting metastasis. These findings highlight that sTIM-3, TGF- β 1, and Vasohibin-1 contribute to OSCC lymph node metastasis via distinct but potentially interactive mechanisms, warranting further mechanistic exploration.

The nomogram model based on these independent predictors showed robust performance in

both cohorts, with AUC values of 0.868 (training) and 0.863 (validation). Calibration curves confirmed good agreement between predicted and actual outcomes, and decision curve analysis verified clinical utility. Compared to existing models, our model strikes a balance between predictive performance and clinical feasibility. For example, Shen et al. developed a model with an AUC of 0.8736 [43], but it relies on intraoperative or postoperative pathological indicators such as lymphovascular invasion, which limits its application in pre-treatment risk stratification. Wang et al.'s model, which integrates molecular markers (CDKN2A, PLAU) and clinicopathological factors, achieved an AUC of 0.807 [44], yet the detection of molecular biomarkers requires specialized equipment and professional expertise, restricting its accessibility in primary care settings. Xu et al. constructed a model combining metabolic variables (SUVmax of lymph nodes from 18F-FDG PET/CT) and pathological variables (T stage), with an AUC of 0.871 in the training cohort and 0.809 in the validation cohort [45]; however, 18F-FDG PET/CT is an expensive and specialized imaging modality that is not readily available in all healthcare institutions, especially in resource-limited regions. In contrast, our model utilizes easily detectable serum biomarkers and basic clinical indicators, enabling early identification of high-risk patients before surgery and facilitating its integration into routine clinical practice. Limitations in comparative

analysis exist due to the heterogeneity and inconsistent reporting of study details in existing literature-many published models lack clear documentation of variable overlap, sample size, or external validation status, which hinders a fully objective and comprehensive assessment of our model's innovative value relative to the broader field. Nevertheless, the model's reliance on accessible and cost-effective variables ensures its practicality for pre-treatment risk assessment in clinical settings.

This study also has several limitations that should be acknowledged. First, it adopts a single-center retrospective design, which may introduce selection bias and thus limit the generalizability of the results to other populations. Second, although SCC-Ag, sTIM-3, TGF- β 1, and Vasohibin-1 were identified as independent predictors alongside clinical stage, the specific molecular mechanisms underlying how these markers regulate OSCC lymph node metastasis remain poorly understood, requiring in-depth investigation. Third, the model has not undergone external validation, and its applicability across different ethnic groups or clinical settings needs further evaluation. These limitations highlight the need for future research to address these gaps and enhance the reliability and utility of the model.

To overcome the aforementioned limitations and build on the current findings, future research should focus on several key directions. Prospective, multicenter studies with larger sample sizes are essential to reduce selection bias and improve the generalizability of the model. Conducting external validation in diverse populations will help verify the model's stability and applicability across different clinical contexts. Additionally, mechanistic studies are warranted to elucidate the interaction networks among sTIM-3, TGF- β 1, and Vasohibin-1, which may reveal novel therapeutic targets for inhibiting OSCC lymph node metastasis. By addressing these aspects, future research can further advance our understanding of OSCC metastasis and translate these findings into improved clinical strategies.

Conclusion

Clinical stage, SCC-Ag, sTIM-3, TGF- β 1, and Vasohibin-1 are independent predictors of lymph node metastasis in OSCC. The nomogram model constructed from these indicators exhibits

excellent discriminative power, calibration accuracy, and clinical utility, effectively identifying high-risk patients for OSCC lymph node metastasis. This study provides a solid basis for the early assessment of lymph node metastasis risk in OSCC patients and the formulation of personalized treatment plans, ultimately holding the potential to improve patient prognosis and clinical outcomes.

Disclosure of conflict of interest

None.

Address correspondence to: Li Fan, Department of Head and Neck Surgery, Shanxi Provincial Cancer Hospital, Taiyuan 030000, Shanxi, China. Tel: +86-18734816800; E-mail: 18734816800@163.com

References

- [1] Zhao B, Li Z and Li R. Exosomes in oral squamous cell carcinoma: functions, challenges, and potential applications. *Front Oncol* 2025; 14: 1502283.
- [2] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209-249.
- [3] Li H, Zhang Y, Xu M and Yang D. Current trends of targeted therapy for oral squamous cell carcinoma. *J Cancer Res Clin Oncol* 2022; 148: 2169-2186.
- [4] Miura KI, Otsuru M, Fukushima H, Omori K, Naruse T, Umeda M and Yamada T. Clinical study on postoperative treatment for patients at high risk of oral squamous cell carcinoma recurrence. *Cureus* 2025; 17: e78395.
- [5] Yang L, Zhang S, Li J, Feng C, Zhu L, Li J, Lin L, Lv X, Su K, Lao X, Chen J, Cao W, Li S, Tang H, Chen X, Liang L, Shang W, Cao Z, Qiu F, Li J, Luo W, Gao S, Wang S, Zeng B, Duan W, Ji T, Liao G and Liang Y. Diagnosis of lymph node metastasis in oral squamous cell carcinoma by an MRI-based deep learning model. *Oral Oncol* 2025; 161: 107165.
- [6] Altuwaijri AA, Aldrees TM and Alessa MA. Prevalence of metastasis and involvement of level IV and V in oral squamous cell carcinoma: a systematic review. *Cureus* 2021; 13: e20255.
- [7] Lu K, Ma H, Wang T, Huang Y and Ru M. The diagnostic value of soluble TIM-3 in oral squamous cell carcinoma. *Future Oncol* 2022; 10: 2217.
- [8] Liu L, Zhong X, Zhong Y and Li L. Recent advances in biomarker detection of oral squa-

- mous cell carcinoma. *Front Oncol* 2025; 15: 1597086.
- [9] Bailly C, Thuru X, Goossens L and Goossens JF. Soluble TIM-3 as a biomarker of progression and therapeutic response in cancers and other of human diseases. *Biochem Pharmacol* 2023; 209: 115445.
- [10] Wuerdemann N, Pütz K, Eckel H, Jain R, Wittekindt C, Huebbers CU, Sharma SJ, Langer C, Gattenlöhner S, Büttner R, Speel EJ, Suchan M, Wagner S, Quaas A and Klusmann JP. LAG-3, TIM-3 and VISTA expression on tumor-infiltrating lymphocytes in oropharyngeal squamous cell carcinoma-potential biomarkers for targeted therapy concepts. *Int J Mol Sci* 2020; 22: 379.
- [11] Zizzari IG, Di Filippo A, Scirocchi F, Di Pietro FR, Rahimi H, Ugolini A, Scagnoli S, Vernocchi P, Del Chierico F, Putignani L, Ruggetti A, Marchetti P, Nuti M, Botticelli A and Napoletano C. Soluble immune checkpoints, gut metabolites and performance status as parameters of response to nivolumab treatment in NSCLC patients. *J Pers Med* 2020; 10: 208.
- [12] Wang J, Xiang H, Lu Y and Wu T. Role and clinical significance of TGF- β 1 and TGF- β R1 in malignant tumors (Review). *Int J Mol Med* 2021; 47: 55.
- [13] Weber M, Ries J, Braun K, Wehrhan F, Distel L, Geppert C, Lutz R, Kesting M and Trumet L. Neoadjuvant radiochemotherapy alters the immune and metabolic microenvironment in oral cancer-analyses of CD68, CD163, TGF- β 1, GLUT-1 and HIF-1 α expressions. *Cells* 2024; 13: 397.
- [14] Liu S, Han B, Sun M, Wang J, Sun Y and Wang Y. Significance of vasohibin 1 in cancer patients: a systematic review and meta analysis. *J Cancer Res Ther* 2022; 18: 567-575.
- [15] Lefebvre F and Giorgi R. A strategy for optimal fitting of multiplicative and additive hazards regression models. *BMC Med Res Methodol* 2021; 21: 100.
- [16] Al-Moraissi EA, Marwan H, Elayah SA, Traxler-Weidenauer D, Paraskevopoulos K, Amir Rais M and Zimmermann M. Sentinel lymph node biopsy versus elective neck dissection in management of the clinically negative (cN0) neck in patients with oral squamous cell carcinoma: a systematic review and meta-analysis. *J Craniomaxillofac Surg* 2024; 52: 141-150.
- [17] Wu YX, Cheng HY, Jia J, Chen GL and Yu ZL. Distinctive lymph node metastasis characteristics and predictive model for young oral squamous cell carcinoma patients under 30. *J Craniomaxillofac Surg* 2025; 53: 1267-1274.
- [18] Duvernay J, Garreau B, Dubreuil PA, Bondaz M, Majoufre C and Schlund M. Lymph node metastasis in level IIb neck dissection for clinically node-negative oral squamous cell carcinoma patients: an 11-year retrospective study. *Int J Oral Maxillofac Surg* 2025; 54: 577-580.
- [19] Jagadeesan D, Sathasivam KV, Fuloria NK, Balakrishnan V, Khor GH, Ravichandran M, Solyappan M, Fuloria S, Gupta G, Ahlawat A, Yadav G, Kaur P and Hussein B. Comprehensive insights into oral squamous cell carcinoma: diagnosis, pathogenesis, and therapeutic advances. *Pathol Res Pract* 2024; 261: 155489.
- [20] Tehzeeb H, Hande A, Patil S, Sonone A, Pakhale A and Chavhan A. Correlation of clinical and pathological TNM staging with histopathological grading in oral squamous cell carcinoma. *Cureus* 2024; 16: e60912.
- [21] Dong L, Xue L, Cheng W, Tang J, Ran J and Li Y. Comprehensive survival analysis of oral squamous cell carcinoma patients undergoing initial radical surgery. *BMC Oral Health* 2024; 24: 919.
- [22] Struckmeier AK, Buchbender M, Agaimy A and Kesting M. Prevalence and implications of bilateral and solely contralateral lymph node metastases in oral squamous cell carcinoma. *Clin Oral Investig* 2024; 28: 267.
- [23] Lin W, Huang M, Zhang Z, Chai T, Chen S, Gao L, Lin J and Kang M. A retrospective study of the relationship between the pathologic subtype and lymph node metastasis of lung adenocarcinomas of ≤ 3 cm diameter. *Medicine (Baltimore)* 2020; 99: e21453.
- [24] Manickasamy MK, Vishwa R, BharathwajChetty B, Alqahtani MS, Abbas M and Kunnumakara AB. Cytokine symphony: deciphering the tumor microenvironment and metastatic axis in oral cancer. *Cytokine Growth Factor Rev* 2025; 85: 1-10.
- [25] Casalone V, Bellomo SE, Berrino E, Bo S, Favaro E, Mellano A, Fenocchio E, Marchiò C and Sapino A. Clinical significance of preoperative nutrition and inflammation assessment tools in gastrointestinal cancer patients undergoing surgery: a retrospective cohort study. *Front Nutr* 2025; 12: 1551048.
- [26] Tang Q, Li X and Sun CR. Predictive value of serum albumin levels on cancer survival: a prospective cohort study. *Frontiers in Oncology* 2024; 14: 1323192.
- [27] Wu YH, Lin PY, Yang JH, Kuo YS and Wu YC. Serum levels and positive rates of tumor biomarkers in oral precancer patients. *J Formos Med Assoc* 2021; 120: 1324-1331.
- [28] Liang H, Zheng Q, Lu J, Li Z, Cai T, Han H, Zhou F, Qin Z, Yao K and Ye Y. Serum cyfra21-1 is a new prognostic biomarker of penile squamous cell carcinoma. *BMC Cancer* 2024; 24: 1240.
- [29] Pourmir I, Benhamouda N, Tran T, Roux H, Pineau J, Gey A, Munoz A, Mabrouk N, Epailard N, Verkarre V, Vano YA, Tartour E and

- Oudard S. Soluble TIM-3, likely produced by myeloid cells, predicts resistance to immune checkpoint inhibitors in metastatic clear cell renal cell carcinoma. *J Exp Clin Cancer Res* 2025; 44: 54.
- [30] Berg J, Halvorsen AR, Bengtson MB, Lindberg M, Halvorsen B, Aukrust P, Helland Å and Ueland T. Circulating T cell activation and exhaustion markers are associated with radiation pneumonitis and poor survival in non-small-cell lung cancer. *Front Immunol* 2022; 13: 875152.
- [31] Peng Y, Zhang C, Rui Z, Tang W, Xu Y, Tao X, Zhao Q and Tong X. A comprehensive profiling of soluble immune checkpoints from the sera of patients with non-small cell lung cancer. *J Clin Lab Anal* 2022; 36: e24224.
- [32] Wang Q, He Y, Li W, Xu X, Hu Q, Bian Z, Xu A, Tu H, Wu M and Wu X. Soluble immune checkpoint-related proteins in blood are associated with invasion and progression in non-small cell lung cancer. *Front Immunol* 2022; 13: 887916.
- [33] Yegin ZA, Can F, Aydın Kaynar L, Gökçen S, Eren Sadioğlu R, Özkurt ZN and Karacaoğlu Ö. Pre-transplant sTIM-3 levels may have a predictive impact on transplant outcome in acute leukemia patients. *Hematology* 2020; 25: 125-133.
- [34] Chaurasia A, Brigi C, Daghrery A, Asa'ad F, Spirito F, Hasuie A, González-Alva P, Kojic DD, Ünsal RBK and Sivaramakrishnan G. Tumor-associated macrophages in oral squamous cell carcinoma. *Oral Dis* 2025; 31: 1546-1563.
- [35] Khatib TO, Pedro BA, Bombin S, Matsuk VY, Robinson IE, Webster SF, Marcus LJ, Summerbell ER, Tharp GK, Knippler CM, Bagchi P, Kowalski-Muegge J, Johnston HR, Ghalei H, Vertino PM, Mouw JK and Marcus AI. TGF- β 1-mediated intercellular signaling fuels cooperative cellular invasion. *Cell Reports* 2025; 44: 115315.
- [36] Sun CP, Bai Y, Jiang JQ and Wu JL. Effects of laparoscopic radical surgery in the treatment of colorectal cancer and correlations of VEGF and TGF- β 1 with prognosis. *Am J Transl Res* 2021; 13: 12887-12896.
- [37] Elahi M and Rakhshan V. MED15, transforming growth factor beta 1 (TGF- β 1), Fc γ RIII (CD16), and HNK-1 (CD57) are prognostic biomarkers of oral squamous cell carcinoma. *Sci Rep* 2020; 10: 8475.
- [38] Koyanagi T, Saga Y, Takahashi Y, Tamura K, Takahashi S, Taneichi A, Takei Y, Mizukami H and Fujiwara H. Forced vasohibin-1 expression increases paclitaxel sensitivity of ovarian cancer by inhibiting microtubule activity. *Cancer Rep* 2024; 7: e70100.
- [39] Kobayashi H, Kosaka T, Mikami S, Kimura T, Hongo H, Kosugi M, Sato Y and Oya M. Vasohibin-1 expression as a biomarker of aggressive nature in ductal adenocarcinoma of the prostate: a retrospective cohort study at two centres in Japan. *BMJ Open* 2021; 11: e056439.
- [40] Sano R, Kanomata N, Suzuki S, Shimoya K, Sato Y, Moriya T and Shiota M. Vasohibin-1 is a poor prognostic factor of ovarian carcinoma. *Tohoku J Exp Med* 2017; 243: 107-114.
- [41] Takahashi Y, Saga Y, Koyanagi T, Takei Y, Machida S, Taneichi A, Mizukami H, Sato Y, Matsubara S and Fujiwara H. Vasohibin-1 expression inhibits advancement of ovarian cancer producing various angiogenic factors. *Cancer Sci* 2016; 107: 629-637.
- [42] Sato Y. Double-face of vasohibin-1 for the maintenance of vascular homeostasis and healthy longevity. *J Atheroscler Thromb* 2018; 25: 461-466.
- [43] Shen H, Zhang T and Wang S. A prediction model for lymph node metastasis of oral squamous cell carcinoma based on multiple risk factors. *Clin Exp Dent Res* 2024; 10: e70046.
- [44] Wang S, Li T, Liu H, Wei W, Yang Y, Wang C, Li B, Han Z and Feng Z. A combined prediction model for lymph node metastasis based on a molecular panel and clinicopathological factors in oral squamous cell carcinoma. *Front Oncol* 2021; 11: 660615.
- [45] Xu F, Peng L, Feng J, Zhu X, Pan Y, Hu Y, Gao X, Ma Y and He Y. A prediction model of nodal metastasis in cNO oral squamous cell carcinoma using metabolic and pathological variables. *Cancer Imaging* 2023; 23: 34.