# Pathological tumor volume as a simple quantitative predictive factor of survival in oral squamous cell carcinoma

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**Abstract.** Oral squamous cell carcinoma (OSCC) is the eighth most common type of cancer in the world. Knowledge of prognostic factors of survival in OSCC is key. Several clinical and pathological prognostic factors have been investigated to develop a prognostic model of survival for patients with oral cancer. The present study focused on the association between pathological tumor volume (PTV) and overall survival time in patients with OSCC, regardless of cervical nodal status. The present study was a prospective study and covered 65 consecutive patients who received surgical treatment for oral cancer. The PTV was calculated according to dimensions of the postoperative specimen. Other pathological parameters as perineural and perivascular tumor spreading and extra-nodular propagation were also determined. The data were analyzed using the IBM SPSS 25.0 software. Cox PH regression model was built to analyze association between the PTV and survival time. Survival time was defined as the period from surgery to a target event or last contact. The results of the present study showed that PTV >4.24 $cm^3$ was significantly associated with shorter overall survival time in patients with OSCC. The PTV value was higher in patients with metastasis and in patients with higher pathological tumor and node stage. In conclusion, PTV was an important pathological prognostic factor for survival in patients with OSCC.

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*Abbreviations:* CT, computed tomography; OSCC, oral squamous cell carcinoma; PTV, pathological tumor volume; RTV, radiological tumor volume; PD-L1, Programmed death-ligand 1

*Key words:* oral squamous cell carcinoma, prognostic, tumor volume, tumor-node-metastasis, survival

## Introduction

Oral squamous cell carcinoma (OSCC) is the eighth most common type of cancer in the world and >177,000 individuals die from oral carcinoma annually (1,2). In Europe, this malignancy ranks 11th in terms of mortality rate, with a cumulative annual incidence of 18.2 in men and 4.9 in women OSCC has a high recurrence rate and is prone to metastasis (3). Treatment of OSCC include surgical treatment, chemotherapy and radiotherapy. Other treatment modalities are considered as immunotherapy and biological therapy (4). The 5-year overall survival rate of OSCC is estimated to be ~54.5% (3). The 5-year overall survival rate in earlier stage is 55-60% while in patients with advanced disease is 30-40% (4). Predicting the clinical course of patients with OSCC at the early stage of the disease is difficult due to the possibility of metastasis. Therefore, identifying novel prognostic factors associated with survival in these patients is of importance. Pathological tumor volume (PTV) is quantitative factor of OSCC which could be interesting for survival prediction. The cut-off value of PTV can indicate significance od this factor in survival patients with OSCC. The tumor-node-metastasis (TNM) system is the most common system of tumor classification used to estimate prognosis (1,5). This staging system refers to the superficial tumor dimension and depth of tumor invasion (1,5). Several clinical and pathological prognostic factors have been investigated to develop a prognostic model of survival for patients with oral cancer. For example, the 8th edition of the American Join Community for cancer included depth of primary tumor invasion and extracapsular extension as prognostic criteria (1,5). The maximal diameter of tumor which is used in this staging is not volumetric measure and therefore it does not determine the tridimensional extend of tumor. Tumor volume can be determined by different methods using imaging scans or by measuring the surgical specimen. Pathological tumor volume (PTV), a quantitative prognostic factor that refers to the 3D nature of the tumor, is associated with patient survival (6). PTV is determined for pathological tissue samples derived from surgically resected primary tumors by measuring the tumor diameter (6). Although the association between PTV and survival of

patients with tongue carcinoma has been previously reported, the association between PTV and other clinical outcomes (occurrence of recurrence and local and regional metastasis) in such patients remains elusive (7,8).

Therefore, the present study aimed to investigate the association between PTV and overall survival in patients with OSCC regardless of cervical nodal status and the cut-off values of PTV.

## Materials and methods

*Patients*. The prospective study included 65 consecutive male (n=53) and female (n=12) patients with age range 38-83 years, who were surgically treated for oral cancer between January 2013 and December 2015 at the Clinic for maxillofacial surgery University Clinical Center in Novi Sad Vojvodina, Serbia. The follow-up period for each patient was 5 years from the date of surgery. The present study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Medicine University of Novi Sad (Novi Sad, Serbia). All patients provided written informed consent for all examinations and treatments. Oral cancer was diagnosed based on anamnesis, physical examination and tumor biopsy. The inclusion criteria were as follows: i) Newly pathohistologically diagnosed patients of any sex with untreated resectable OSCC; ii) >18 years of age and iii) no radiologically diagnosed distant metastasis. The exclusion criteria were as follows: i) Patients with a history of any malignancy other than basal cell carcinoma of the skin; ii) patients with recurrent oral carcinoma; iii) prior completion of one or more courses of therapeutic irradiation; vi) patients with autoimmune disease or HIV infection and v) patients with distant metastasis. All patients included in the study were also HPV-negative. Tumor size and TNM stage were determined via clinical examination, biopsy and head, neck and thorax computed tomography (CT). The patients were treated according to their TNM status determined by the clinical findings and CT results. Treatment approaches included radical tumor resection and neck dissection.

Surgically resected tumors were fixed on a styrofoam surface and marked according to localization in the mouth or neck. Following tumor diameter measuring, the tissue was fixed with 10% formalin and embedded in paraffin, followed by staining with hematoxylin and eosin (H&E) solution. Fixation of the specimen started as soon as the resection operation finished. The bottle containing the specimen was kept at room temperature at all times. The specimen was fixed for 6-24 h. After cutting the tissue, specimens were moved to the automated procession procedure in the Epredia™ Excelsior™ AS Tissue Processor (Thermo Fisher Scientific, Inc.) following the recommended procedure. Staining with H&E lasted for 90 min at 21˚C. The tissue sectioning was performed using an Accu-Cut SRM 200 Rotary Microtome (Sakura Finetek USA, Inc.) or a Microtome HM355S (Thermo Fisher Scientific, Inc.). The thickness of the microscopic sections was 5-7 $\mu$m. The primary antibodies used were as follows: anti-GAPDH (1:1,000; cat. no. 48245; Thermo Fisher Scientific, Inc.), FLEX Monoclonal Mouse Anti-Human p63 Protein Clone DAK-p63 R, Ready-to-Use (cat. no. IR 662; Dako; Agilent Technologies, Inc.), FLEX Monoclonal Mouse Anti-Human Ki-67 Clone MIB-1, Ready-to-Use (cat. no. IR626; Dako; Agilent Technologies, Inc.). Epredia™ Dewax and HIER Buffer L (cat. no. TA-999-DHBM; X15; Thermo Fisher Scientific, Inc.) was used as the blocking reagent at 65˚C then heated to 98˚C for 10 min before being allowed to cool to 65˚C using the LAB Vision™ PT Module (Thermo Fisher Scientific, Inc.). An Espedia Autostainer 360 (Thermo Fisher Scientific, Inc.) and Bench Mark GX (Roche Tissue Diagnostics) were used for staining. Incubation with primary antibodies was at room temperature for 10 min. The following reagents were used: Epredia™ DAB Quanto Detection System (cat. no. 12674017; Fisher Scientific; Thermo Fisher Scientific. Inc.), Ultra view Universal DAB Detection Kit (cat. no. 760-500; Roche Tissue Diagnostics; Roche Diagnostics, Ltd.) and Erpedia™ Ultra Vision Detection System HRP (cat. no. 12684017; Fisher Scientific; Thermo Fisher Scientific, Inc.). Both primary antibodies were conjugated with the Epredia™ Primary Antibody Amplifier Quanto and Epredia™ HRP Polymer Quanto supplied with the aforementioned Epredia™ DAB Quanto Detection System.

The postoperative pathological examination was performed by an experienced pathologist. The light microscope Zeiss Axio Scope A1 was used (representative images are shown in Figs. 1 and 2).

Based on the histopathological findings, patients were treated with the appropriate chemotherapy and radiotherapy regimen <6 weeks following surgical resection. Low-risk patients were treated with external radiotherapy of 60-70 Gy in 30-35 fractions for 6-7 weeks. High-risk patients with positive margins, >2 positive nodes or extracapsular spreading received concurrent chemotherapy with intravenous bolus of 100 mg/m$^3$ cisplatin for 3 weeks combined with radiotherapy of 60-70 Gy in 30-35 fractions for 6-7 weeks. At the end of the chemotherapy/radiotherapy cycles, the patients were monitored every 2 months for 2 years, every 6 months for the next 2 years and then every year after that. CT of the head, neck and chest were routinely performed to detect any recurrence or distant metastasis.

*Determination of pathological parameters*. The pathological parameters determined for surgical specimens were as follows: i) Largest (diameter A) and smallest transverse diameter (diameter B) of the tumor were measured on an unfixed macroscopic sample; ii) tumor thickness (vertical distance from the surface of the tumor to the point of its deepest invasion, cm) was measured via microscopic examination with an accuracy of 0.1 mm; iii) the invasion depth (vertical distance between basal membranes of the closest intact mucosa and the deepest point of tumor invasion) was also determined via microscopic examination with an accuracy of 0.1 mm and was expressed in mm; iv) pathological volume of the primary tumor was calculated using the following formula: PTV (cm$^3$)=$\pi$/6 x diameter A x diameter B x tumor thickness; v) radiological tumor volume (RTV) was determined by measuring tumor dimensions from the CT scan using the following formula: RTV (cm$^3$)=$\pi$/6 x diameter A x diameter B x tumor thickness (8) and vi) presence of perineural, perivascular and perinodular spread, dysplasia and positive resection margins determined by pathologist.

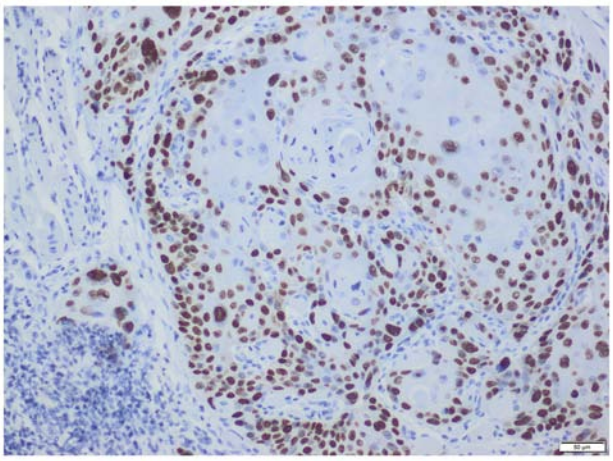

Figure 1. Immunohistochemical staining of p63 to show infiltration of squamous cell carcinoma in the deeper part of the tongue muscle tissue (magnification, x100).

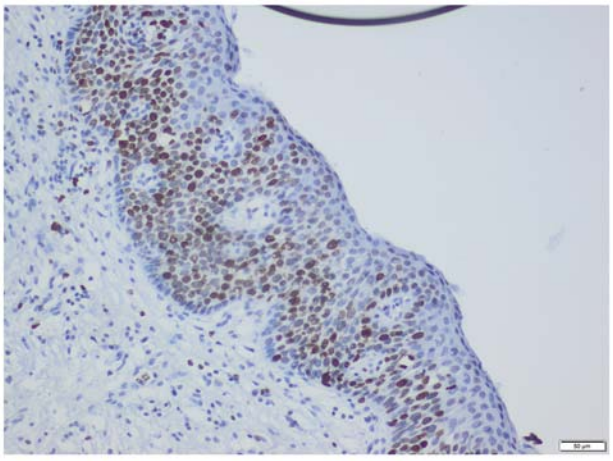

Figure 2. Immunohistochemical staining of Ki 67 to show the enlarged number of proliferative cells in the mucosal squamous epithelium in the carcinoma vicinity (magnification, x100).

*Statistical analysis*. Data were analyzed using SPSS 25.0 software (IBM Corp.). Data are presented as the mean and standard deviation. The association between PTV, according to the pathohistological findings, and the clinicopathological features of patients, including sex, tumor site, pathological tumor (T) and node (N) stage status, dysplasia, margins of resection, perineural and perivascular spreading and metastasis, were analyzed by Mann-Whitney U or Kruskal-Wallis test. Mann-Whitney test was used to detect statistically significant differences between two independent samples. Kruskal-Wallis was used to detect differences in medians between more than two independent samples. Spearman correlation was used to asses the relationship between variables that do not follow normal distribution. To determine the optimal cut-off value of tumor volume, to divide cases into groups, receiver operating characteristic (ROC) curve analysis was performed using the Youden index (9). The Kaplan-Meier method and log-rank test were performed to evaluate differences in the survival distribution for the calculated cut-off values. $\chi^2$ was used to compare differences in the survival between the two cut-off groups. Two multiple Cox proportional hazards regression models were constructed to explore the association between the tumor-dependent variables and survival time. Omnibus test was performed to assess the validity of the models. The regression analysis results are expressed as a P-value, hazard ratio and confidence interval (CI) for hazard ratio [95 CI for Exp(B)]. Survival time was defined as the period between surgery and a target event (such as death) or last contact. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Demographic and tumor-associated features*. A total of 65 patients with a mean age of 59.6±9.4 years were included in the study. 81.5% of patients were males. Of all patients, 53.8% had no metastases. With regards to localization of the tumor in the oral cavity, a primary tumor was located on the tongue of 32 patients, on the floor of the mouth in 22 patients, on the hard palate in 4 patients, on the gingiva in 4 patients and on the buccal mucosa in 3 patients (Table I).

*Association between PTV and clinicopathological features of patients*. The association between PTV and clinicopathological characteristics of patients with OSCC is presented in Table II. PTV was significantly higher in patients with metastasis and those with higher pathological T and N status. However, PTV was significantly decreased in patients who survived compared with those who died. No significant association was observed between PTV and the other parameters examined. There was no significant difference between RTV calculated using CT examination and PTV calculated on the surgically resected specimen which indicate that pretreatment tumor could be determinated using CT. These two quantities are positively correlated ($\rho=0.4$), meaning that an increase in one volume shows an increment in the other volume.

The association between survival rate of patients and their clinicopathological parameters is shown in Table III. Statistically significant associations were observed between survival time and tumor metastasis, as well as with the pathological T and N status.

*Cox model survival analysis*. Two Cox proportional hazard regression models were constructed to identify potential predictors of survival in patients with OSCC (Table IV). There were no associations between risk factors for oral cancer analyzed. In the first model, four predictors were analyzed, RTV, PTV, perineural spreading and perivascular spreading. In the second model, RTV, perineural spreading and perivascular spreading were also used as predictors. However, in the second model, tumor dimensions were used instead of PTV. Omnibus tests of model coefficients revealed that both models were statistically significant [model 1, $\chi^2(4)=13.617$; model 2, $\chi^2(7)=19.070$]. However, the only significant variable in model 1 was PTV, indicating that the higher the PTV, the less likely the patient was to survive. In the second model, none of the indicators were statistically significant.

*ROC survival analysis*. ROC curve analysis was performed to evaluate the significant predictors in the Cox model and

Table I. Demographic data and tumor-associated characteristics of 65 patients.

| Characteristic | Value |
|---|---|
| Mean age ± SD, years | 59.65±9.42 |
| Male, n (%) | 53 (81.50) |
| Without metastases, n (%) | 35 (53.80) |
| Censored (patient died), n (%) | 28 (43.10) |
| Median life expectancy following surgery, months; IQ | 41;64 |
| Dysplasia present, n (%) | 26 (40.00) |
| Positive margins of resection, n (%) | 6 (9.20) |
| Tumor location, n (%) | |
| Tongue | 32 (49.20) |
| Floor of the mouth | 22 (33.80) |
| Hard palate | 4 (6.20) |
| Gingiva | 4 (6.20) |
| Buccal mucosa | 3 (4.60) |
| RTV mean ± SD, $cm^3$ | 6.71±5.23 |
| PTV mean ± SD, $cm^3$ | 6.67±7.77 |
| PT diameter A mean ± SD, cm | 3.19±1.42 |
| PT diameter B mean ± SD, cm | 2.23±1.00 |
| PT thickness mean ± SD, cm | 1.34±0.51 |
| PT depth of tumor invasion | 9.17±5.46 |
| DOI,mean ± SD, mm | |
| T stage[a], n (%) | |
| T1 | 8 (12.30) |
| T2 | 29 (44.60) |
| T3 | 27 (41.50) |
| T4 | 1 (1.50) |
| N stage[a], n (%) | |
| N0 | 35 (53.80) |
| N1 | 7 (10.80) |
| N2 | 15 (21.10) |
| N3 | 8 (12.30) |
| Perineural spreading, n (%) | 23 (35.40) |
| Perivascular spreading, n (%) | 10 (15.40) |
| Perinodalar spreading, n (%) | 11 (16.90) |

[a](1,5). IQ, interquartile range; N, node; T, primary tumor; PTV, pathological tumor volume (according to pathohistological findings); RTV, radiological tumor volume (according to computed tomography findings).

Table II. Association between pathological tumor volume and clinicopathological parameters.

| Parameter | Median | Interquartile range | P-value |
|---|---|---|---|
| Sex | | | 0.859 |
| Male | 3.930 | 43.86 | |
| Female | 3.210 | 10.24 | |
| Dysplasia | | | 0.904 |
| Absent | 3.925 | 33.39 | |
| Present | 3.310 | 42.78 | |
| Margins of resection | | | 0.196 |
| Negative | 3.271 | 43.86 | |
| Positive | 8.308 | 14.95 | |
| Perineural spreading | | | 0.105 |
| Absent | 2.826 | 23.18 | |
| Present | 4.710 | 43.86 | |
| Perivascular spreading | | | 0.964 |
| Absent | 3.533 | 3.533 | |
| Present | 3.925 | 33.39 | |
| Metastasis | | | 0.006 |
| Absent | 2.355 | 23.45 | |
| Present | 6.018 | 43.02 | |
| Survival | | | 0.005 |
| Survived | 2.944 | 23.45 | |
| Censored[a] | 6.411 | 43.02 | |
| Tumor site | | | 0.341 |
| Tongue | 4.710 | 33.39 | |
| Floor of the mouth | 3.336 | 43.21 | |
| Other | 3.140 | 12.19 | |
| Pathological tumor classification[b] | | | 0.001 |
| T1 | 0.994 | 1.47 | |
| T2 | 2.512 | 11.78 | |
| T3 | 8.373 | 42.78 | |
| T4 | 16.485 | 0.00 | |
| Pathological node classification[b] | | | 0.005 |
| N0 | 2.355 | 23.45 | |
| N1 | 3.140 | 5.23 | |
| N2 | 6.280 | 14.76 | |
| N3 | 12.560 | 42.39 | |

[a]Patients who did not survive by the end of the study. [b](1,5).

to determine the optimal cut-off volume for identifying pathological tumors. PTV exhibited a good capacity to discriminate pathological from non-pathological tumors (area under the curve=0.70608; Fig. 3). The performance of PTV was determined by calculating sensitivity, specificity and positive and negative predictive values (Table V). Sensitivity and specificity values of PTV were 68 and 70%, respectively, while the negative and positive predictive values were 63 and 74%, respectively. Furthermore, ROC curve analysis (Fig. 3) and Youden index showed that the optimal cut-off volume of PTV was 4.24 $cm^3$ (Youden index, 0.3812).

*Kaplan-Meier survival analysis*. Log-rank test was performed to determine the differences in the survival distribution for different PTV cut-off values (Table VI). The analysis showed that differences in survival distribution between the two groups (PTV ≤4.24 and PTV >4.24) were statistically significant

Table III. Association between pathological tumor volume and clinicopathological parameters.

| Parameter | Survived, % | Censored[a], % | P-value |
|---|---|---|---|
| Sex | | | 0.913 |
| Male | 56.6 | 43.4 | |
| Female | 58.3 | 41.7 | |
| Dysplasia | | | 0.919 |
| Absent | 56.4 | 43.6 | |
| Present | 57.7 | 42.3 | |
| Margins of resection | | | 0.719 |
| Negative | 57.6 | 42.4 | |
| Positive | 50.0 | 50.0 | |
| Perineural spreading | | | 0.567 |
| Absent | 59.5 | 40.5 | |
| Present | 52.2 | 47.8 | |
| Perivascular spreading | | | 0.631 |
| Absent | 58.2 | 41.8 | |
| Present | 50.0 | 50.0 | |
| Metastasis | | | 0.011 |
| Absent | 67.6 | 32.4 | |
| Present | 35.7 | 64.3 | |
| Tumor site | | | 0.334 |
| Tongue | 65.6 | 34.4 | |
| Floor of the mouth | 45.5 | 54.5 | |
| Other | 54.5 | 45.5 | |
| Pathological tumor classification[b] | | | 0.002 |
| T1 | 75.0 | 25.0 | |
| T2 | 69.0 | 31.0 | |
| T3 | 40.7 | 59.3 | |
| T4 | 0.0 | 100.0 | |
| Pathological node classification[b] | | | 0.002 |
| N0 | 71.4 | 28.6 | |
| N1 | 71.4 | 28.6 | |
| N2 | 46.7 | 53.3 | |
| N3 | 0.0 | 100.0 | |

[a]Patients who did not survive by the end of the study. [b](1,5).

[$\chi^2(1)=6.318$]. Patients with PTV >4.24 $cm^3$ exhibited a significantly shorter survival time compared with those with PTV ≤4.24 $cm^3$. Kaplan-Meier survival curves were plotted based on the PTV cut-off (Fig. 4).

## Discussion

The results of the present study showed that PTV >4.24 $cm^3$ was significantly associated with shorter overall survival time in patients with OSCC. Two models were constructed using Cox proportional hazard regression models. In the first model, PTV was used but in the second model, pathohistological parameters used to calculate PTV (such as tumor dimensions and thickness) were used. The first model exhibited an improved prognostic value compared with the second model. In addition, PTV was higher in patients with metastatic OSCC and in those with higher pathological T and N status. Additionally, PTV was notably decreased in patients who survived compared with those who died. However, no significant differences were observed between PTV and the other parameters examined. Of patients included in the present study, 43.1% died. This was consistent with a previous study demonstrating that the average 5-year mortality rate in patients with oral cancer is 45.5% (3).

Several pathological parameters serve a key role in the prognosis of OSCC. The significant association between tumor invasion depth and thickness with cancer prognosis has been reported in several studies, suggesting that tumor invasion depth could be used in TNM staging of OSCC (6,7). Additionally, tumor size, grade, thickness and invasion depth as well as perineural and perivascular spreading, bone infiltration and surgical margins status are significant factors associated with overall survival in patients with oral cancer (8). Therefore, these parameters have been incorporated into guidelines for the treatment of oral carcinoma (10,11). Nevertheless, investigating these parameters is of great importance as prognostic stratification serves a critical role in treatment planning.

PTV, calculated by 3D measurement of the primary tumor, is a significant pathological parameter in several types of cancer (laryngeal carcinoma, tumors of pancreas, liver tumors) (10). Tumors of higher T status exhibit larger tumor volume. However, this is not always applicable. The lower tumor volume observed in some cases of T3 and T4 OSCC could be due to the amorphic nature and irregular shape of the tumor (6). Tumor volume has been also associated with survival time (11). Mucoyama *et al* (8) demonstrated that PTV >18 $cm^3$ is significantly associated with a shorter survival time and suggested that PTV, calculated using the same formula used in the present study, serves as an important pathological parameter of OSCC localization. In addition, Mücke *et al* (12) showed that increased PTV is notably associated with shorter survival time in patients with tongue squamous cell carcinoma. Therefore, the present study hypothesized that PTV may be significantly associated with overall survival in patients with OSCC, which includes patients with squamous cell carcinoma of the entire oral cavity. The results showed that in a clinical sample of 65 patients, PTV value >4.24 $cm^3$ was significantly associated with shorter overall survival time. This finding indicated that patients with PTV >4.24 $cm^3$ should undergo more frequent postoperative examinations and CT scans to improve overall survival rate.

The multivariate Cox regression analysis results in the present study suggested that PTV may be considered as a significant prognostic factor. It has also been reported that PTV is associated with pathological T and N status, thus indicating that it may be significantly associated with the survival of patients with OSCC (6). Therefore, in addition to tumor invasion depth and tumor thickness, PTV may be used in OSCC staging (13,14). However, in order to obtain a more optimal prognostic model, it should be investigated whether the prognostic value of PTV improves when combined with

Table IV. Variables in the Cox proportional hazard regression models.

A, Model 1

| Variable | P-value | Hazard ratio | 95% CI for Exp(B) | |
|---|---|---|---|---|
| | | | Lower | Upper |
| RTV | 0.284 | 1.039 | 0.969 | 1.114 |
| PTV | 0.013 | 1.057 | 1.012 | 1.105 |
| Perineural spreading | 0.941 | 1.035 | 0.422 | 2.534 |
| Perivascular spreading | 0.977 | 0.984 | 0.313 | 3.095 |

B, Model 2

| Variable | P-value | Hazard ratio | 95% CI for Exp(B) | |
|---|---|---|---|---|
| | | | Lower | Upper |
| RTV | 0.294 | 1.041 | 0.966 | 1.122 |
| PH tumor size A | 0.233 | 1.261 | 0.861 | 1.845 |
| PH tumor size B | 0.223 | 1.470 | 0.790 | 2.735 |
| PH tumor thickness | 0.421 | 0.643 | 0.220 | 1.884 |
| PH depth of tumor invasion | 0.804 | 1.014 | 0.908 | 1.133 |
| Perineural spreading | 0.709 | 1.200 | 0.460 | 3.129 |
| Perivascular spreading | 0.688 | 0.770 | 0.215 | 2.759 |

CI, confidence interval; Exp(B), odds ratio; PH, pathohistological; PTV, pathological tumor volume (according to pathohistological findings); RTV, radiological tumor volume (according to computed tomography findings).

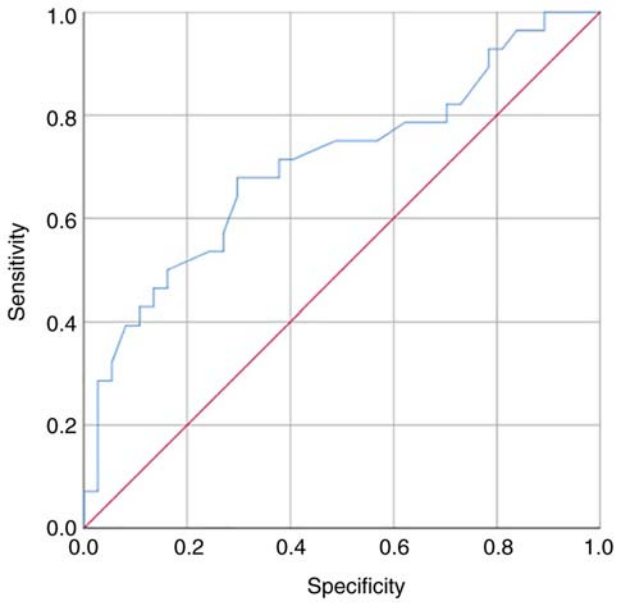


Figure 3. ROC curve. The blue line represents the ROC curve, the red line denotes the non-predictive 45˚ line that corresponds to random classifiers. ROC, receiver operating characteristic.

Table V. Pathological tumor volume performance measures.

| Performance measure | Value | Lower limit | Upper limit |
|---|---|---|---|
| Sensitivity | 0.679 | 0.476 | 0.841 |
| Specificity | 0.703 | 0.530 | 0.841 |
| Positive predictive value | 0.633 | 0.452 | 0.813 |
| Negative predictive value | 0.743 | 0.555 | 0.866 |
| Positive likelihood ratio | 2.282 | 1.308 | 3.984 |
| Negative likelihood ratio | 0.457 | 0.257 | 0.815 |

that of other prognostic factors, such as those associated with patient and tumor characteristics as well as treatment.

The present study had certain limitations. Firstly, the study size was relatively small. Therefore, a larger multicenter study with a larger sample size should be performed in the future to verify the significant prognostic value of PTV. The results of a multicenter study could further support the role of PTV as a promising prognostic parameter in OSCC staging. Secondly, in the present study PTV values were determined based on data obtained from postoperative samples. These values may differ from those obtained during an *in vivo* assessment due to tissue shrinkage during the sample drying procedure. Advances in imaging technology facilitate preoperative determination of tumor volume, particularly in patients unwilling to undergo surgery. Therefore, F-fludeoxyglucose positron emission tomography in combination with CT may be a good basis for diagnostic assessment of patients with head and neck tumors (15). In addition, RTV of OSCC can be measured

Table VI. Calculated means for the survival time.

| PTV | Mean estimate | Standard error | 95% Confidence interval | |
|---|---|---|---|---|
| | | | Lower bound | Upper bound |
| ≤4.24 | 50.421 | 4.012 | 42.558 | 58.285 |
| >4.24 | 35.142 | 4.976 | 25.390 | 44.895 |
| Overall | 43.796 | 3.274 | 37.378 | 50.214 |

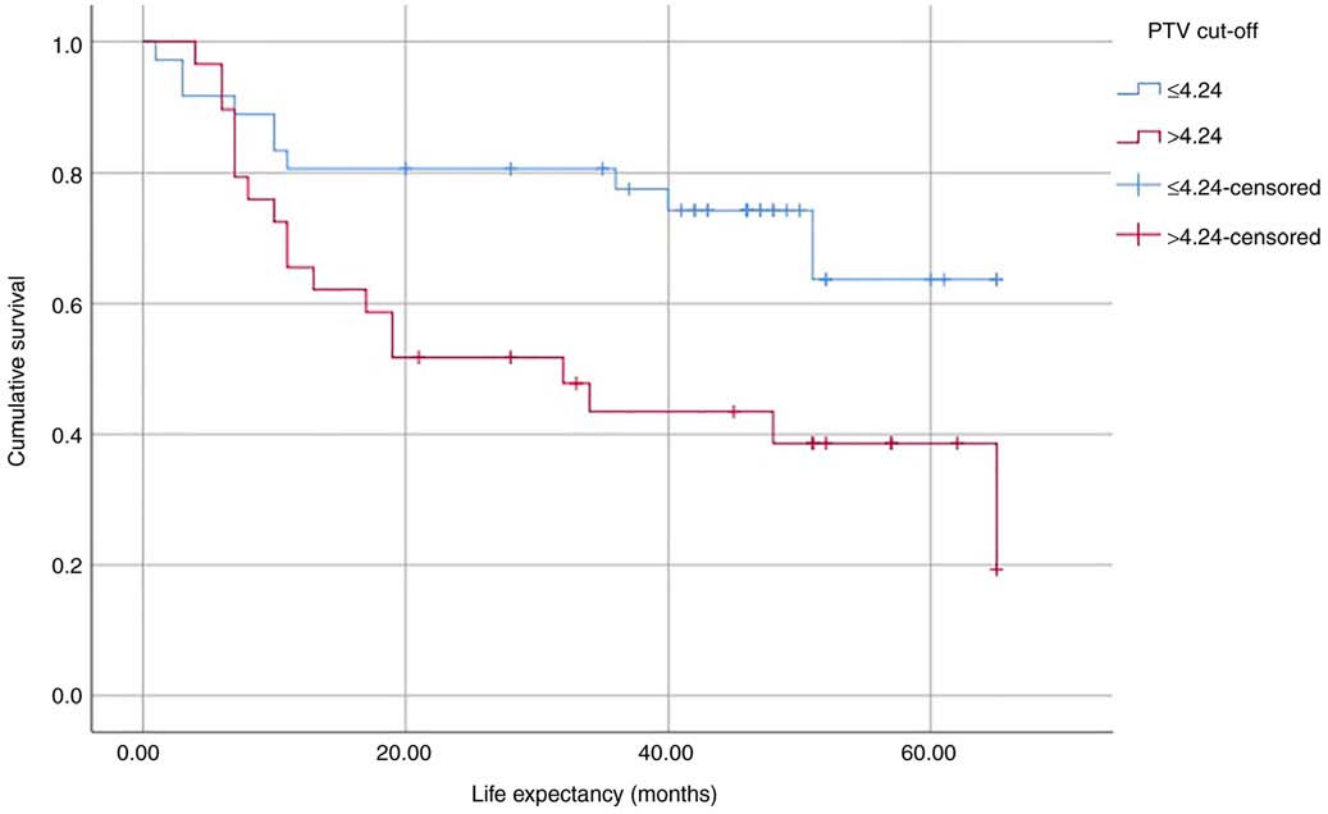


Figure 4. Kaplan-Meier survival analysis. Censored patients were those who did not survive. PTV, pathological tumor volume.

before surgical treatment, thus helping surgeons in planning the resection surgery (16,17). Tumor volume in OSCC is an important tumor metric, since it is associated with OSCC outcome as well as tumor size, TNM stage, marginal status and perineural spreading (17). Programmed death-ligand 1 (PD-L1) expression calculated using tumor proportion score was not determined in the present study as this is not a standard for oral cancer diagnosis in Serbia. Due to the data type in the present study, differences in PTV between surviving and deceased patients were assessed. Survival status was binary; therefore, correlation was not a measure of dependence.

Disease staging in patients with OSCC is of importance since it allows the classification of patients into prognostic groups and the application of appropriate therapies, while also facilitating communication between physicians involved in treatment and the familiarization of patients with the prognosis (18,19). TNM classification plays an important role in planning treatment of patients with cancer, predicting survival and establishing treatment protocols (1,5). The system is serviceable and practical. However, it leaves room for expansion of additional variables. In OSCC, T stage is based on the maximum size of tumor and invasion depth. However, other parameters such as tumor thickness and PTV could also be included in T status, thus providing a more accurate determination of the 3D image of the tumor (20,21). The head and neck regions contain several anatomical structures that can affect the clinical picture of the disease. Nodal status is also considered a significant parameter associated with patient survival. However, other pathological and radiological parameters could also exhibit prognostic value and could therefore be applied in OSCC staging (6). The significance of several simple, quantitative prognostic factors such as tumor thickness and invasion depth has been already reported (13,20). However, applying additional quantitative and qualitative factors, could make OSCC staging easier and more precise for clinicians, inferring a need for further research in this field (21). The present study suggested that PTV contributed to improved disease staging and survival

prognosis. Moreover, the improved survival of patients with low PTV highlighted the importance of detecting OSCC at an earlier stage of the disease when tumor volume is decreased. Tumor volume has a notable effect on overall survival of patients with oral cancer, so it may be considered an essential factor in the selection of appropriate treatment options for each patient (7).

In conclusion, the present study demonstrated that PTV, as a 3D tumor measure, was a significant factor associated with survival time of patients with OSCC. PTV value >4.24 $cm^3$ was significantly associated with shorter overall survival time in patients with OSCC, suggesting that PTV exerted a significant prognostic value in OSCC, which could be applied in disease staging. 3D analysis of the tumor using PTV may complement the T staging system, while tumor sphericity determination may lead to an improved determination of OSCC in the future. In the future, it would be beneficial to fit the Cox proportional hazard model on a dataset with a larger sample size and extended set of prognosis predictors (such as PD-L1 expression, Epidermal Growth Factor Receptor gene polymorphism or ZEB-1 expression).

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**Availability of data and materials**

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

**Authors' contributions**

IM, SM, AK, MPI and NV contributed to conception and design of the study. IM, SM, AK and MPI were involved in the surgical treatment of patients. IM was involved in the statistical analysis of data and writing the manuscript. AK, NV, JN and AT made critical revisions to the manuscript. NV performed pathohistological examination. AS, JN and AT contributed to data collection, analysis and interpretation of data. AS performed the radiological measurements. AK and NV confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Ethics Committee of the Faculty of Medicine University of Novi Sad (Novi Sad, Serbia; approval no. 01-39/112/1) and all patients provided written informed consent for all the examinations and treatments.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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