Addition of the tumour–stroma ratio to the 8th edition American Joint Committee on Cancer staging system improves survival prediction for patients with oral tongue squamous cell carcinoma

The addition of tumour-stroma ratio to the 8th AJCC staging system improves survival prediction in oral tongue squamous cell carcinoma

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Abstract

Background: One of the objectives of current researches is to customise the treatment of cancer patients. This objective requires stratification of patients based on the most significant prognostic factors. The aim of this study was to evaluate the prognostic value of tumour-stroma ratio (TSR), defined as the proportion of tumour cells relative to surrounding stroma, in patients with Oral Tongue Squamous Cell Carcinoma (OTSCC) and to develop a prognostic nomogram based on the most significant clinico-pathological features.

Methods: Clinico-pathological data of 211 patients treated at "Ospedali Riuniti" General Hospital (Ancona, Italy) for OTSCC were collected. 139 patients were re-staged according to the 8th AJCC edition. Evaluation of TSR was performed on haematoxylin and eosin-stained slides and correlation with survival outcomes evaluated. In addition, aiming to integrate the independent value of TSR with the 8th edition of AJCC, a prognostic nomogram for OTSCC has been developed.

Results: OTSCC with low TSR (i.e. high proportion of stroma and low proportion of tumour cells) showed to have a negative prognostic value in terms of disease-specific survival with a hazard ratio (HR) of 1.883 and 95% confidence interval (CI) of 1.033-3.432 (*P*=0.039) and overall survival (HR=1.747, 95% CI 0.967-3.154; *P*=0.044) independently from other histological and clinical parameters. For the cohort of 139 patients re-staged according to the 8th AJCC edition, variables correlating with a poor prognosis were: TSR, perineural invasion, and gender. The nomogram built on these parameters showed a good predictive capacity, over performing the 8th AJCC staging system in stratifying disease-specific survival in OTSCC.

Conclusions: Including TSR in the predictive model could improve risk stratification of OTSCC patients and aid in treatment decision.

INTRODUCTION

Oral cancer accounts for 2% of all cancers and is associated with a high mortality rate. Oral squamous cell carcinoma (OSCC) represents more than 90% of oral cancers with tongue as the most common involved site [1]. Despite recent advances in diagnostic techniques, the prognosis of OSCCs is poor and often unpredictable [2]. Oral tongue is the most commonly involved site, accounting for about 40% of all OSCC cases [3]. Some studies have shown that oral tongue squamous cells carcinoma (OTSCC) presents different molecular features and clinical behaviour compared to OSCC from other subsites of the oral cavity [4, 5]. Although these findings cannot be considered conclusive, studying independent cohorts of OTSCCs could eliminate the presence of an "anatomical bias" [6-8]. Surgical resection of primary OTSCC is still considered the gold standard of treatment [9]. Unfortunately, OTSCC is characterised by a high rate of recurrence, which strongly decreases patients' survival rates. Currently, there are no reliable biomarkers available in clinical practice that can stratify patients according to prognosis. The American Joint Committee on Cancer (AJCC) staging system is the most widespread used tool for prognostic stratification of OSCC patients [10]. Recently, the 8th edition of AJCC staging system has been released and new parameters have been added to stratify OSCC patients, such as depth of invasion (DOI) and extra-nodal extension (ENE) [11]. This new classification system for OSCC results to perform better than the previous one; however, accumulating evidences show that the predictive capability still need to be improved to obtain a robust prognostic classification [12].

Although it is widely accepted that the prognosis is closely associated with the tumour staging at the time of diagnosis, there are other factors that might influence the course of the disease. Several genetic, epigenetic, and histological biomarkers have been proposed in order to identify the aggressive tumours with the aim to provide more personalised cancer therapies [13, 14]. Furthermore, several researches conducted in recent years have increasingly shown that tumour microenvironment

plays a role in cancer progression [15]. In particular, tumour stroma basically consists of the non-malignant cells of the tumour microenvironment (including cancer-associated fibroblasts, innate and adaptative immune cells, micro-vessels, and extracellular matrix) all of which are interposed between the malignant cells and normal host tissues [16]. The formation of tumour stroma is a complex process induced by tumour-host interactions and seems to influence tumour invasiveness, suggesting a prognostic value comparable to that of other tumour features [17].

The proportion between neoplastic cells and tumour-associated stroma in tumour tissue has been defined as Tumour-Stroma Ratio (TSR). A low TSR, that implies a relatively high quantity of stroma, has been shown to be an adverse prognostic factor for several tumours [18-20]. A recent study on a cohort of early-stage OTSCCs showed that the stroma-rich group had a worse prognosis compared to the stroma-low group [21].

The aim of this study was to analyse if the TSR could be a predictive factor of prognosis for OTSCC, considering both the early and late-stage cancers, classified according to either 7th and 8th editions of the AJCC staging system [11, 22]. On the basis of the performance of TSR in the multivariate analysis, a prognostic nomogram aiming to predict the prognosis of OTSCC patients was developed.

MATERIAL AND METHODS

Study population

The cohort included in this retrospective study consists of randomly selected patients with OTSCC. All the patients were treated with a curative intent at the Department of Maxillofacial Surgery, "Ospedali Riuniti" General Hospital (Ancona, Italy), between 1997 and 2014. The clinical and pathological data were collected from the archives of the Institute of Pathology, Marche Polytechnic University, Italy.

Inclusion criteria were: (a) primary OTSCC; (b) age over 18 years; (c) no preoperative chemo- or radiation therapy nor adjuvant chemotherapy; (d) no human papilloma virus (HPV) infection (assessed by using HPV 16-specific fluorescence in situ hybridisation and $p16^{Ink4a}$-specific immunohistochemistry); (e) follow-up data of at least 3 years for alive patients. Exclusion criteria were: (a) OTSCC cases that also involve other anatomical sites, and where the exact site from which the tumour originated could not be identified; (b) relapsed or secondary primary OTSCC; (c) OTSCC patients with immediate postoperative death.

The staging classification was revised by 2 expert pathologists (C.R. and M.M.) according to both the 7th and 8th editions of the AJCC Cancer Staging Manual [11, 22] and the 4th edition of the World Health Organization (WHO) classification of Head and Neck tumours [23].

Follow-up data were updated by a single surgeon (P.B.), blinded to clinical and pathological data and group allocation. Clinical endpoints examined were disease-specific survival (DSS), overall survival (OS) and disease-free survival (DFS). Follow-up time was calculated from the date of surgical operation to the date of recurrence (for DFS), to the date of death due to cancer (for DSS) or death for any cause (OS), or the date of the last visit. Informed consent was obtained from all included patients, and the study was conducted in accordance with the "Ethical Principles for Medical Research Involving Human Subjects" statement of the Helsinki Declaration [24]. This study received

ethical approval from the institutional review board of Marche Polytechnic University, Italy (CERM 2019-308). The study was conducted according to REMARK checklist [25] and transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement.

Histopathologic evaluation

Haematoxylin and eosin (HE)-stained sections (4 µm) obtained from formalin-fixed, paraffin-embedded blocks of the primary tumour specimens. Two experienced pathologists (C.R. and M.M.) independently performed the histological evaluation, blinded to the clinical and pathological data. TSR was visually assessed as previously reported [26]. Slides were selected from the most invasive part of the OTSCC (i.e. the same slides routinely used to assess the T status). Then, the invasive area appearing to have the highest percentage of desmoplastic stroma was jointly identified by the two pathologists by conventional light microscopy at low magnification. Subsequently, each pathologist independently evaluated the same area at high magnification (200×) and a single high-power field was selected and scored for each case. In particular, only those areas where tumour cell nests are present at all borders of the selected image-field were evaluated (Figure 1). The percentage of stroma tissue was manually estimated per tenfold, from 0% to 100%, per image-field. The mean value of the percentages provided by both pathologists was used as final score.

For statistical analysis, patients were subsequently dichotomised in stroma-rich group, defined by the presence of high proportion of stroma and low proportion of tumour cells (TSR < 50%) and stroma-poor group, consisting in low proportion of stroma and high proportion of tumour cells (TSR ≥ 50%). Specific areas, such as skeletal muscle tissue, salivary gland tissue, large nerve bundles, large blood vessels or necrotic tissue have been left out of the microscopic scoring field whenever possible. If this was not possible, these areas have been ignored for scoring. Each pathologist gave a judgement on the belonging of each specimen to a single class of TSR (< 50% or ≥ 50%). The independent scores were used to assess the degree of agreement between the observers by calculating a Cohen's

Kappa. Subsequently a joint session with a third author (G.T.) was scheduled in order to give a final judgment of allocation in cases of disagreement between the two pathologists. Regarding Perineural Invasion (PNI), it was evaluated according to Liebig et al. [27]. Briefly, the presence of PNI was reported when cancer cells were identified in any of the 3 layers of the nerve sheath and/or tumour was in close proximity to the nerve, involving more than one-third its circumference

Statistical analysis

All the statistical analyses were performed by using the following tools: SPSS 21.0 (IBM Corporation, Chicago, IL, USA) and Stata 16.0 (StataCorp LLC, USA). Primary endpoint was to detect any significant difference in the survival rate between stroma-poor (high TSR) and stroma-rich (low TSR) cases. In order to evaluate the correlation of the TSR with clinico-pathological variables, Spearman rank correlation analysis was performed entering TSR either as continuous or categorical variable. Normal distribution of variables was explored through Shapiro-Wilk normality test and the Kolgomorov-Smirnoff test. The non-parametric test of Mann-Whitney was used in order to investigate the difference in expression between TSR groups. Univariate Cox Regression analysis was used to estimate the association among variables and survival outcomes (DSS, OS and DFS). In addition, a multivariate Cox proportional hazard model was built in order to assess the association among predictive variables (TSR, Stage, WHO Grade, PNI, Age, and Gender) and their influences on the prognostic outcomes, the proportional hazard assumption was checked with the test of nonzero slope in a generalised linear regression of the scaled Schoenfeld residuals on time (*estat* command on Stata 16.0). The nomogram was generated using the nomocox package in Stata 16.0 [28], internal validation was performed through estimation of bootstrap adjusted c-index performing 1000 bootstrap iterations. In addition, the performances of the new model were compared with those of 8th edition of AJCC staging system alone by means of Harrell c-index, Akaike information criterion and time-dependent ROC curves at 1, 3 and 5-year follow-up.

RESULTS

Demographic and clinico-pathological variables

A total of 211 samples from OTSCC patients admitted and treated at Department of Maxillofacial Surgery, “Ospedali Riuniti” General Hospital, Ancona, Italy, in the period between 1997 and 2014 were included in this study. All these patients had been staged according to the 7th AJCC staging system. In addition, 139 OTSCC patients were "restaged" according to the 8th AJCC staging system based on the information available about DOI and ENE. The retrospective analysis revealed a mean follow-up time of 50.4 ± 36.8 months (range 3-120). Furthermore, 44.5% (94/211) died from OTSCC, 6.2% (13/211) died for cancer-unrelated reasons and 49.3% (104/211) were alive at the last follow-up date. Results showed that 32.7% (69/211) had a loco-regional recurrence, defined as disease recurrence in the local site or regional lymph node but not secondary primary tumour, with a mean follow-up time of 52.6 ± 37.5 months (range 8-120). A good interobserver agreement (Cohen κ=0.807) was obtained by the independent evaluation of TSR by two authors, suggesting a good reproducibility in the scoring of this histological variable. More details about clinico-pathological data of patients included into the cohort are reported in Table 1. No significant relationship was found between TSR (both as continuous and categorical variables) and other clinico-pathological variables (WHO Grade, PNI, Gender, pT Stage, and pN Stage). PNI significantly correlated with pN 7th AJCC edition (ρ= 0.319, P = 0.003) and pT 7th AJCC edition (ρ= 0.256, P < 0.001), in addition a correlation between pN and pT 7th AJCC edition was found (ρ= 0.186, P = 0.008) (Table 2).

Prognostic value of TSR

Survival analysis was first performed for the whole group of 211 OTSCC patients according to the 7th AJCC edition, and then repeated for the subgroup of 139 OTSCC restaged according to the 8th AJCC edition. Stage, WHO Grade, TSR, PNI, and male gender were significantly correlated with a worse DSS and OS and were included in the multivariate analysis. The results of the Cox proportional hazard model showed that patients with stroma-rich (low TSR) OTSCC had a significant worse DSS

with a hazard ratio (HR) of 1.598 and 95% confidence interval (CI) of 1.034 to 2.745 (*P* = 0.036), and poor OS (HR = 1.685, 95% CI 0.998-2.560; *P* = 0.051) than stroma-poor (high TSR) tumours (Table 3, Figure 2). Among the predictors included into the multivariate analysis Stage, PNI (Figure 3), male gender, and advanced age showed significant association with the survival outcomes, whereas the WHO Grade was not significantly correlated with DSS or OS. It is noted that age was entered as a continuous variable, finding an association between the progressive increase of the patients' age and worse survival outcome. The same multivariate model was built using DFS as outcome; however, none of the predictors were significant. On the basis of these results, we performed multivariate survival analysis for the subgroup of 139 patients re-staged according to the 8th AJCC edition. Since WHO Grade did not correlate with survival analysis into the multivariate models, we decided to exclude it from the subsequent analyses. Thus; Cox Proportional Hazard Models were built for DSS, OS, and DFS including Stage (8th AJCC edition), Gender, Age, PNI, and TSR. Also, for this group of patients, in which results were adjusted according to the 8th AJCC Stage, TSR correlated with both DSS (HR = 1.883 95% C.I. 1.033-3.432; *P* = 0.039) and OS (HR = 1.747, 95% CI 0.967-3.154; *P* = 0.044) (Table 4).

Development of the nomogram and measurements of performance

A nomogram showing the probability of 3- and 5-year DSS rate using the Cox Proportional Hazard model is reported in Figure 4. Age was entered into the model as a continuous variable, while 8th AJCC Stage, TSR, PNI and Gender were considered as categorical variables. The total scores generated from the nomogram for each patient showed good values of discrimination (c-index = 0.765). In addition, interval validation was performed through bootstrapping to test for apparent performance optimistic which was therefore excluded (bootstrap c-index = 0.7649). The predictive probabilities of the nomogram were used to tabulate ROC curve for the dichotomous outcome (Dead/Alive) and to find the best threshold value for discriminating between high risk and low risk

patients based on nomogram total score. In particular, using 15.03 as Total Score for categorisation, showed 83.64% of sensitivity and 76.83% of specificity as predictive performance values. Furthermore, the performance of the proposed nomogram was compared to that of the 8th AJCC staging system alone. The nomogram showed either higher values of Harrell c-index (0.765 vs 0.715) and lower of Akaike information criterion (445.528 vs 454.313). In addition, the new model exceeded the AJCC staging system in the analysis of time-dependent ROC curve at 1-year (AUC = 0.821 vs 0.701), 3-year (AUC = 0.771 vs 0.575) and 5-year (AUC = 0.823 vs 0.543) follow-up for DSS.

DISCUSSION

The recent implementation of clinico-pathological parameters of the 8th edition of the AJCC Cancer Staging Manual allows for better stratification of patients with OTSCC [29]. Nevertheless, the prognostic prediction for OTSCC patients is still unsatisfactory, making the search for new prognostic markers necessary [21]. Despite immunohistochemistry and other molecular techniques that are well-established methods to identify new prognostic markers, the variable results and the high cost hinder their usefulness in daily clinical practice [30]. For these reasons, the study of morphological features of tumour tissue could be a valuable source of prognostic information.

Several studies showed that the stromal component of tumours seems to have a high prognostic value for several types of solid malignancies [20]. In particular, the measurement of TSR seems to be a reliable, accurate, and economical method to characterise the role of tumour stroma in tumour progression [26]. Since its first report in colorectal cancer patients in 2007, the measurement of TSR in HE-stained slides demonstrated its robustness and high reproducibility in other solid epithelial tumours [31-34]. In particular, almost all of the studies showed that a low TSR (i.e. the presence of a high proportion of stroma in tumour tissue) is significantly related to poor prognosis in patients with different types of solid tumours [20, 35, 36].

Until now, the role of TSR in head and neck tumours has been little investigated. The first study was conducted in 2014 in a small cohort of patients with nasopharyngeal cancers to investigate the prognostic role of TSR, reporting a significantly worse 5-year OS and DFS in stroma-rich tumours [18]. Recently, Karpathiou et al. explored the possible prognostic role of TSR in laryngeal and pharyngeal cancers [37]. Using two different cut-off points (50% and 30%) to classify the stroma-rich tumours, they found that low TSR was significantly associated with advanced T-stage and poor survival. Regarding the oral cavity, in 2018 a multicenter study was conducted with the aim to evaluate the prognostic value of TSR in early-stage (T1-2N0M0) OTSCC [21]. The results were interesting, showing that low TSR is an independent risk factor for recurrence and cancer-related mortality in patients with early-stage OTSCC. Similarly, another pilot study on a small cohort of OSCC patients showed that stroma-rich tumours had a worse prognosis than stroma-poor cases [38]. In particular, low TSR was related to a significant worse 3-year DFS (44%) than high TSR (69.04%).

The present study is the first to investigate the TSR in OSCC, particularly in OTSCC, using the 8th edition of the AJCC Cancer Staging Manual. Our results are consistent with the orientation of the previously discussed literature. Since the 8th AJCC edition has been recently released, we decided to update our database in accordance with it, considering the parameters introduced by the new classification [39]. Information about ENE could not be retrieved for 72 cases; therefore, a complete reclassification was possible only for 139 samples. Indeed, ENE is now a mandatory parameter for staging OSCC patients with lymph node metastases. Nevertheless, we performed a correlation analysis on this subgroup, confirming what it already found in 7th AJCC edition, namely that TSR directly correlated with both DSS (HR 1.883, 95% CI 1.033-3.432; $P = 0.039$) and OS (HR 1.747, 95% CI 0.967-3.154; $P = 0.044$) also when cases staged according to 8th edition of AJCC.

The PNI, defined as the dissemination of cancer cells in and along nerve bundles, has long been recognised as an indicator of poor prognosis in OSCC patients [40]. Among the advantages of using PNI are the ease of evaluation and the lack of influence from anatomic characteristics of different

oral subsites [41]. Indeed, recent studies suggested PNI as a predictor of decreased 5-year survival in OSCC [42-44]. Although PNI is a well-recognised prognostic factor in OSCC, it is not required for stage grouping in the 8th edition of AJCC staging system [45]. Furthermore, the different prognostic significance of unifocal and multifocal PNI remains unclear. The lack of agreement about the prognostic impact of PNI may be explained by several factors such as the different evaluation methods for assessing PNI or the presence of potential confounders (e.g. the possibility that two foci of PNI may represent two segments of one involved nerve bundle) [46]. For this reason, in the present study we used a dichotomous score (presence/absence of PNI) in our statistical analyses, showing that the correlation between PNI and DSF was significant in 7th AJCC staging system (P = 0.010) but at the limits of significance (P = 0.053) in 8th AJCC staging system. This result is probably due to the reduced sample size (139 cases) after the "restaging" process. Nevertheless, the trend reported here highlights the importance of PNI for risk stratification in OSCC patients.

The gender difference found in survival outcome should be interpreted with caution, since it could be due to a number of biological and behavioural factors. First of all, it has been reported that men are generally less aware of tumour signs and symptoms, partially explain the diagnostic delay and a more advanced stage at diagnosis [47]. Furthermore, several studies have found the presence of an interplay between tumour microenvironment and oestrogen signalling pathway, suggesting a potential role of these hormones in modulating tumour stroma, although this is only a hypothesis which requires further examination [48].

Four variables, including 8th edition AJCC staging, TSR, PNI, and gender, were included in the nomogram for survival prediction in OTSCC patients. Our proposed model was used to estimate 3- and 5-year DSS, providing good stratification of the patients into prognostic groups. In particular, the nomogram exhibited 83.64% of sensitivity and 76.83% of specificity using 15.03 as Total Score for categorisation. Furthermore, the prognostic performance of the developed nomogram demonstrated

powerful predictive value compared with 8th edition AJCC staging [12], encouraging further investigations.

The possibility to implement the TSR in daily practice is supported by other advantages, such as the low inter-observer variation, especially if a cut-off-point of 50% is used. Another advantage is the little extra time required to evaluate TSR. Indeed, as reported by some authors and confirmed in this study, the evaluation of TSR feasible method to perform in daily practice and takes a maximum of 2 minutes [26]. In this study, TSR was analysed by entering this parameter either as continuous or categorical variable, getting the same results. Therefore, by using a dichotomous score (TSR < 50% and ≥ 50%), we obtained a good interobserver agreement (Cohen κ=0.807), suggesting as the assessment of TSR seems to be reproducible among different observers. Indeed, the use of a dichotomous score significantly reduced the confounding effect of tumour heterogeneity of the invasive front. However, in those cases where the value of TSR ranges between 40 to 60%, the presence of a heterogeneous tumour front might affect the categorisation, risking to misinterpret a stroma-poor tumour for a stroma-rich one. Of note, it has been advised to select stroma-rich areas as deeming decisive for the scoring of TSR in such heterogenous tumours in the previous studies [19, 21].

There are several issues that must be considered when measuring TSR. Some of these have been previously mentioned, such as the presence of skeletal muscle tissue, salivary gland tissue, large nerve bundles, large blood vessels or necrotic tissue. These areas must be left out of the microscopic field or ignored for scoring. However, the tongue is a muscular organ and the areas of muscle invasion could make particularly difficult to estimate the percentage of stroma tissue in advanced OTSCCs. In some cases, in particular T3 and T4 cancers, the skeletal muscle invasion could be the main invasion pattern of OTSCC. Although the TSR is evaluated only in the invasive area with the highest percentage of tumour stroma, a possible limitation is that in some tumours this parameter could be difficult to apply. Furthermore, the presence of heavy inflammation could make it particularly

difficult to estimate the percentage of stroma tissue. In these cases, the measurement of TSR may take longer. Indeed, the presence of heavy inflammatory infiltration in the tumour stroma could make the estimate of TSR difficult, but the inflammatory cell component should not be excluded *per se*.

According to some authors, the evaluation of TSR should be used with caution in patients eventually pre-treated with chemo-radiotherapy, due to the altered stromal formation in the tumour microenvironment [26]. However, the recent advances in head and neck imaging could be used in the next future to accurately estimate the TSR through the analysis of intratumoural heterogeneity [49]. Lastly, the reliability of TSR as prognostic parameter in incisional biopsies of OSCC has not yet been studied.

The main limitations of the present investigation are the relatively low sample size and its retrospective nature. Furthermore, lack of information about ENE in 72 cases prevented us from a reclassification of all OTSCC cases from 7th to 8th AJCC edition, limiting the prognostic impact of other clinico-pathological factors considered. Beside this, the obtained results provide important insights about the prognostic significance of histopathologic characteristics. In conclusion, evaluation of TSR is simple, inexpensive and can be used in daily practice. Together with PNI, the TSR can be used to augment risk stratification of OTSCC based on 8th AJCC edition.

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Figure 1. Examples of TSR in HE-stained sections of OTSCC. A and B, correct field of visions: tumour cells are on all four sides (black arrows). C and D, wrong field of vision: tumour cells are only present at two sides (black arrows).

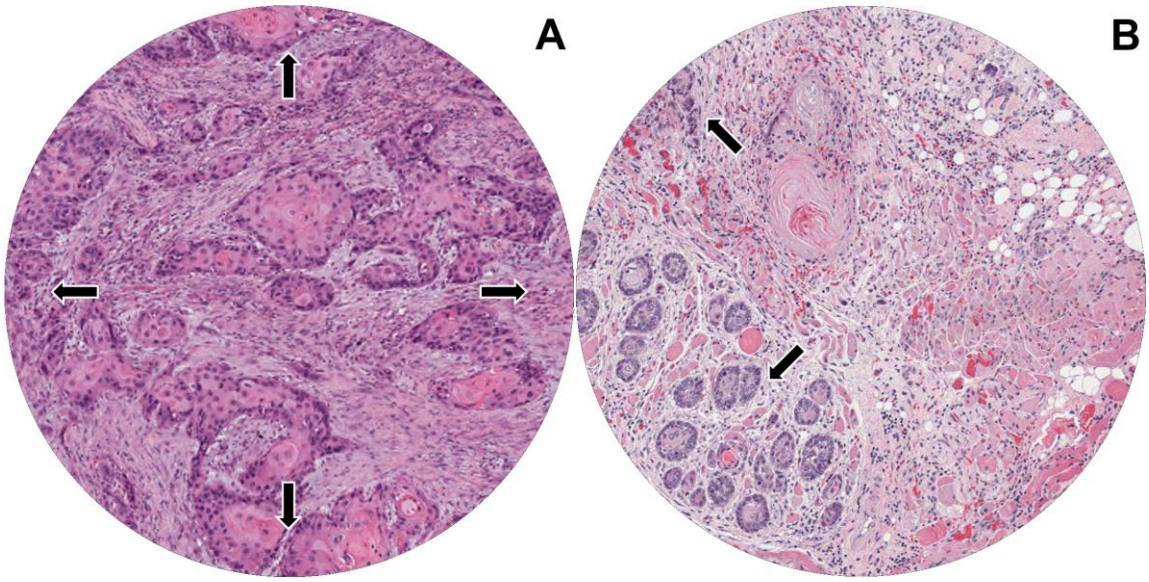


Figure 2. Evaluation of TSR in HE-stained sections of OTSCC. A, B, and C, three examples of stroma-rich tumours. The inset areas of greater magnification showed a TSR <50%. D, E, and F, three examples of stroma-poor tumours. The inset areas of greater magnification showed a TSR ≥50%.

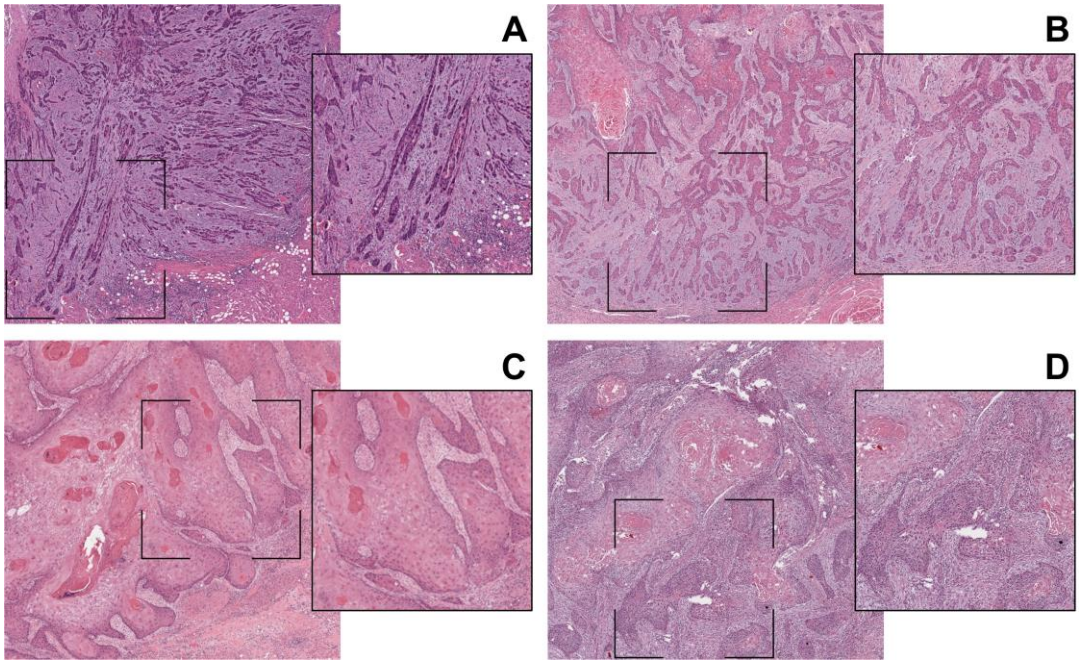


Figure 3. Perineural Invasion in OTSCC: hematoxylin-eosin section showing squamous cell carcinoma encircling the nerve trunk.

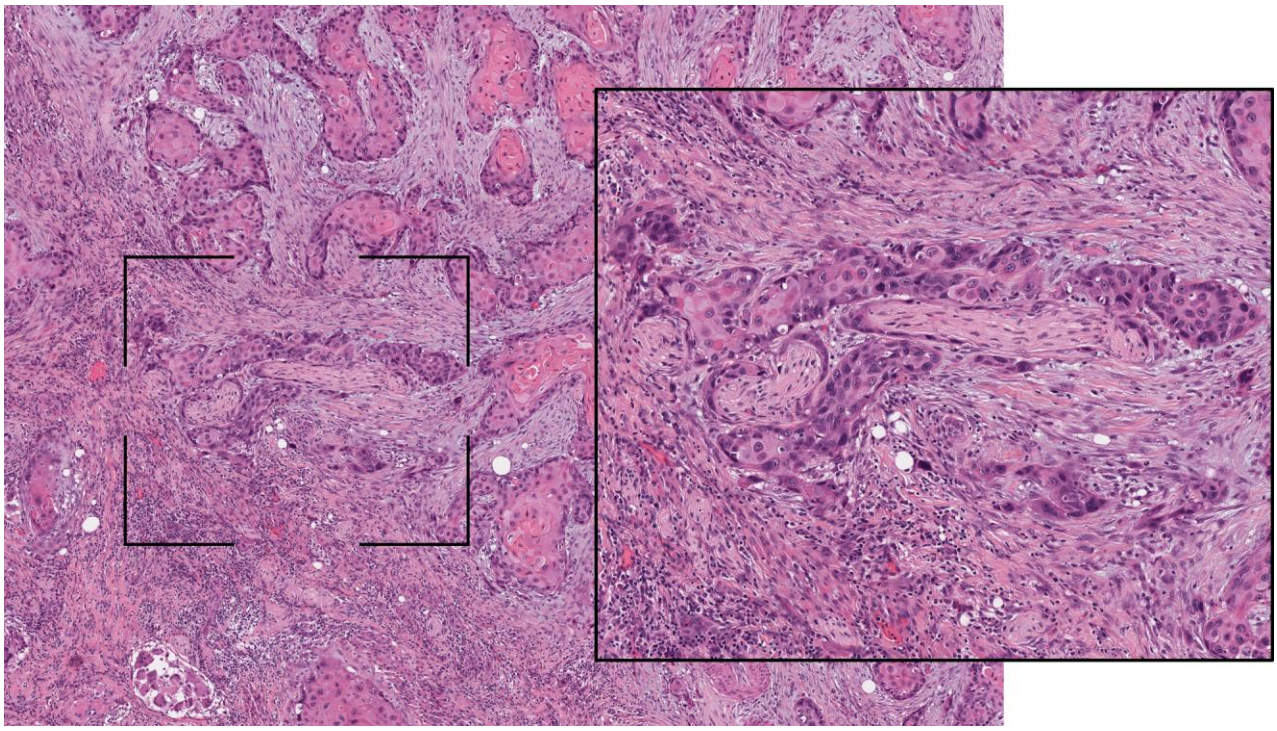
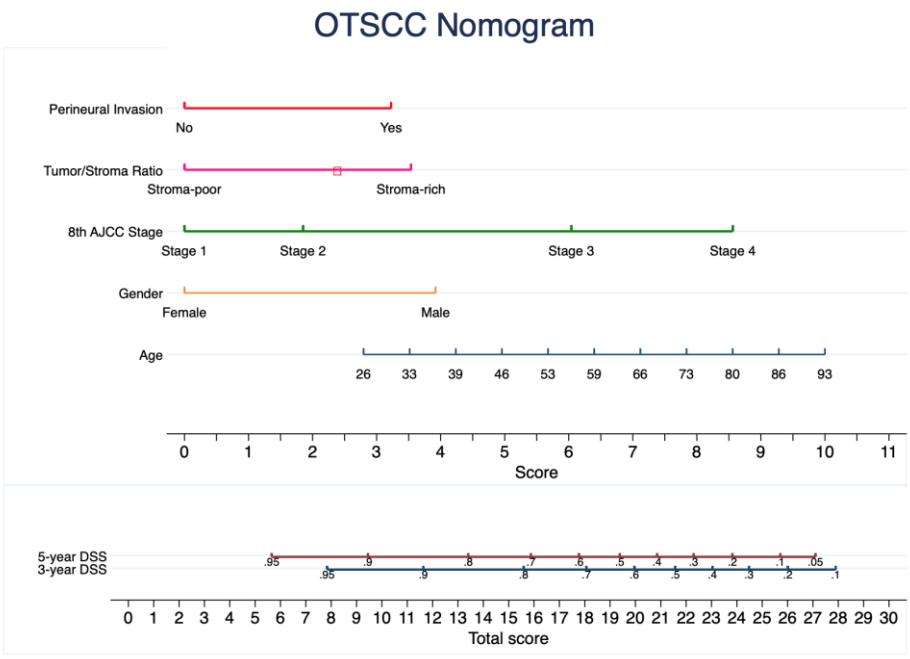


Figure 4. Nomogram developed from the multivariate cox-proportional hazard model including variables: Age, Gender, Perineural Invasion, Stage and TSR.



Clinic-pathological features	AJCC 7th Edition (211 Patients)	AJCC 8th Edition (139 Patients)
Age		
=< 65	111	74
> 65	100	65
Gender		
Male	137	86
Female	74	53
Grade		
1-2	152	97
3	59	42
Stage		
1-2	111	60
3-4	100	79
Status DSS		
Dead	94	57
Alive	117	82
Status DFS		
Relapse	69	46
Disease-Free	142	93

Table 1: Clinicopathological information of patients included in the study. DSS (Disease-Specific Survival), DFS (Disease-Free Survival), AJCC (American Joint Committee on Cancer).

Variable	Age	Gender	Grade	Perineural Invasion	pT(7th AJCC)	pN(7th AJCC)	Stage (7th AJCC)	Tumor/Stroma
Age	ρ = 1	***0,173***	***-0,205***	-0,049	-0,028	-0,047	-0,031	0,008
	p-value = 1	***0,012****	***0,03****	0,487	0,687	0,501	0,658	0,907
Gender		ρ = 1	-0,115	0,097	***-0,138***	-0,103	***-0,166***	-0,085
		p-value = 1	0,104	0,170	***0,045****	0,142	***0,016****	0,220
Grade			ρ = 1	***0,259***	***0,152***	***0,209***	***0,250***	0,082
			p-value = 1	***0,000****	***0,031****	***0,003****	***0,000****	0,248
Perineural Invasion				ρ = 1	**0,256**	***0,215***	***0,335***	0,039
				p-value = 1	***0,000****	***0,003****	***0,000****	0,583
pT(7th AJCC)					ρ = 1	***0,186***	***0,534***	0,104
					p-value = 1	***0,008****	***0,000****	0,133
pN(7th AJCC)						ρ = 1	***0,803***	0,116
						p-value = 1	***0,000****	0,097
Stage (7th AJCC)							ρ = 1	0,124
							p-value = 1	0,073
Tumor/Stroma								ρ = 1
								p-value = 1

Table 2: Spearman rank correlation for variables evaluated into the cohort of 211 patients classified according to the 7th Edition of the AJCC staging. * $p < 0,05$; ** $p < 0,001$

Variables		Disease-Specific Survival		Disease-Free Survival	
		HR 95%(CI) -	*p-value*	HR 95%(CI) -	*p-value*
Stage	1 (ref)	1.00	-	1.00	
	2	1.71 (0.79-3.70)	0.174	0.84 (0.41-1.76)	0.652
	3	1.47 (0.64-3.37)	0.363	1.08 (0.51-2.26)	0.845
	4	2.97 (1.38-6.37)	0.005*	0.90 (0.42-1.89)	0.772
Grade	A (ref)	1.00	-	1.00	-
	B	0.73 (0.38-1.40)	0.343	1.59 (0.70-3.62)	0.270
	C	0.83 (0.40-1.71)	0.613	1.66 (0.66-4.17)	0.283
P.I.	No	1.00	-	1.00	-
	Yes	2.04 (1.19-3.50)	0.010*	1.25 (0.71-2.20)	0.448
Sex	Female	1.00	-	1.00	-
	Male	1.65 (1.00-2.27)	0.028*	1.06 (0.62-1.81)	0.830
TSR	Low	1.00	-	1.00	-
	High	1.68 (1.03-2.75)	0.036*	1.65 (0.92-2.96)	0.111
Age		1.02 (1.00-1.04)	0.033*	1.00 (0.98-1.02)	0.710

Table 3: Cox Regression Hazard Model for Disease-Specific Survival and Disease-Free Survival for the cohort of 211 OTSCC patients staged according to the 7th AJCC edition.

Variables		Disease-Specific Survival		Disease-Free Survival	
		HR 95%(CI) -	*p-value*	HR 95%(CI) -	*p-value*
Stage	1 (ref)	1.00	-	1.00	
	2	1.42 (0.39-5.17)	0.174	0.49 (0.16-1.30)	0.153
	3	3.14 (0.95-10.45)	0.061	1.07 (0.43-2.63)	0.883
	4	5.07 (1.72-14.95)	0.003*	0.72 (0.31-1.65)	0.432
P.I.	No	1.00	-	1.00	-
	Yes	1.84 (0.99-3.42)	0.053	1.24 (0.64-2.41)	0.517
Sex	Female	1.00	-	1.00	-
	Male	2.10 (1.10-4.02)	0.024*	1.31 (0.67-2.58)	0.426
TSR	Low	1.00	-	1.00	-
	High	1.96 (1.09-3.51)	0.025*	2.27 (1.13-4.53)	0.021*
Age		1.02 (0.99-1.04)	0.089	0.99 (0.97-1.02)	0.720

Table 4: Cox Regression Hazard Model for Disease-Specific Survival and Disease-Free Survival for the cohort 137 patients "re-staged" according to the 8th AJCC edition.