doi: 10.5146/tjpath.2013.01144

The Prognostic Value of Tumor-Stroma Proportion in Laryngeal Squamous Cell Carcinoma

Laringeal Skuamöz Hücreli Karsinomlarda Tümör-Stroma Oranının Prognostik Değeri

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ABSTRACT

Objective: Tumor-stroma proportion of tumor has been presented as a prognostic factor in some types of adenocarcinomas, but there is no information about squamous cell carcinomas and laryngeal carcinomas.

Material and Method: Five digital images of the tumor sections were obtained from 85 laryngeal carcinomas. Proportion of epithelial tumor component and stroma were measured by a software tool, allowing the pathologists to mark 205.6 μm^2 blocks on areas as carcinomatous/stromal, by clicking at the image. Totally, 3.451 mm^2 tumor areas have been marked to 16.785 small square blocks for each case.

Results: Median follow up was 48 months (range 3-194). The mean tumor-stroma proportion was 48.63+18.18. There was no difference for tumor-stroma proportion when tumor location, grade, stage and perinodal invasion were considered. Although the following results were statistically insignificant, the mean tumor-stroma proportion was the lowest (37.46±12.49) for subglottic carcinomas, and it was 52.41±37.47, 50.86+19.84 and 44.56±16.91 for supraglottic, transglottic and glottic cases. The tumor-stroma proportion was lowest in cases with perinodal invasion and the highest in cases without lymph node metastasis (44.72±20.23, 47.77±17.37, 50.05±17.34). Tumor-stroma proportion was higher in the basaloid subtype compared with the classical squamous cell carcinoma (53.76±14.70 and 48.63±18.38 respectively). The overall and disease-free survival analysis did not reveal significance for tumor-stroma proportion (p=0.08, p=0.38). Only pathological stage was an independent factor for overall survival (p=0.008).

Conclusion: This is the first series investigating tumor-stroma proportion as a prognostic marker in laryngeal carcinomas proposing a new method, but the findings do not support tumor-stroma proportion as a prognostic marker.

Key Words: Laryngeal neoplasms, Prognosis, Stromal cell, Tumor

ÖZ

Amaç: Tümör-stroma oranı bazı adenokarsinom tiplerinde kötü prognostik faktör olarak tanımlanmış olmakla birlikte skuamöz hücreli karsinomlarda ve özellikle de laringeal karsinomlarda tümör-stroma oranı ile ilgili araştırma bulunmamaktadır.

Gereç ve Yöntem: Seksen beş larenjektomi materyalinin tümörü örnekleyen kesitlerinden 5 dijital görüntü kaydedildi. Tümör-stroma oranı bu görüntülerde bilgisayar programının 205,6 μm^2 büyüklüğünde küçük kareler oluşturması ve patoloğun bilgisayar faresinde sol/sağ tıklama ile her bir alanı tümör ya da stroma olarak seçmesini sağlayan bir bilgisayar yazılım programı ile hesaplandı. Toplamda her olguda 16.785 tık ile 3.451 mm^2 tümör alanı işaretlendi.

Bulgular: Ortalama izlem 48 ay (3-194) ve ortalama tümör-stroma oranı 48,63±18,18 bulundu. Tümör yerleşimi, histolojik derecesi, evresi ve perinodal yayılım ile tümör-stroma oranları arasında anlamlı farklılık saptanmadı. İstatistiksel anlamlı sonuç elde edilmemiş olmakla birlikte ortalama tümör-stroma oranının en düşük izlendiği olgular subglottik yerleşimli olup ortalama oran 37,46±12,49 idi. Ortalama tümör-stroma oranı supraglottik olgularda 52,41±37,47, transglottik yerleşimde 50,86±19,84 ve glottik yerleşimde 44,56±16,91 olarak saptandı. Tümör-stroma oranı perinodal invazyon gösteren olgularda (44,72±20,23) düşük iken, perinodal invazyon bulunmayan (47,77±17,37) ve lenf nodu metastazı olmayanlarda 50,05±17,34 daha yüksek bulundu. Bazaloid skamoz hücreli karsinomlarda tümör-stroma oranı (53,76±14,70), klasik skuamöz hücreli karsinomdan (48,63±18,38) daha yüksek saptandı. Hastalıksız sağ kalım ve toplam sağ kalım ile tümör-stroma oranı arasında ilişki bulunmadı (p=0,08, p=0,38). Sadece patolojik evrenin prognostik bağımsız faktör olduğu saptandı (p=0,008).

Sonuç: Bu çalışma yeni bir yöntem eşliğinde, laringeal karsinomlarda tümör-stroma oranı'nın prognostik değerini araştıran ilk çalışmadır. Ancak bulgular tümör-stroma oranını prognostik bir belirleyici olarak desteklememektedir.

Anahtar Sözcükler: Larinks tümörleri, Prognoz, Tümör, Stromal hücreler

(Turk Patoloji Derg 2013, 29:27-35)

Received : 21.05.2012 **Accepted :** 06.12.2012

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INTRODUCTION

All malignant epithelial tumors composed of epithelial cells are admixed with supportive tissue, named as "stroma" and this special tissue includes vascular-lymphatic channels and inflammatory cells as well as some type of fibrous tissue, so called desmoplasia. Desmoplastic stroma is composed of activated fibroblast-like cells and new extracellular matrix, induced by invasion of malignant cells (1,2) and today, it is known that the tumor stroma has an important role in tumor progression, invasion and metastasis (1,3,4). Tumor-stroma ratio or tumor-stroma proportion (TSP) has been presented as a prognostic factor in some types of carcinomas, including prostate, colorectal, esophageal and breast adenocarcinomas (5-11). Additionally, desmoplasia is presented as a poor prognostic factor in colon and lung adenocarcinoma (12-14) and in squamous cell carcinomas (SCC) of the skin and the desmoplastic variant of skin SCC is known as a very aggressive tumor (15-18). There is no information about TSP of SCC and laryngeal carcinomas, in particular.

Among all cancers, carcinoma of the larynx accounts for 2.2% in men and 0.4% in women, with an increase at the latter group, probably due to changing habits of smoking (19). Most of the laryngeal carcinomas originate from the supraglottic and glottic region. Although glottic carcinomas frequently remain localized for a long time, supraglottic and subglottic carcinomas tend to spread into the pre-epiglottic region and pyriform sinus as well as base of the tongue and subglottic SCC may spread to the thyroid gland, hypopharynx, esophagus and tracheal wall. Many unfavorable prognostic factors are defined for laryngeal carcinomas, including advanced stage, subglottic localization, high microscopic grade, high number and size of involved lymph nodes, and presence of extranodal extension, epidermal growth factor receptor expression, tumor budding and DNA aneuploidy (20-25). In this study the prognostic value of TSP is investigated in laryngeal SCC for the first time, in a series of patients treated with surgery and radiotherapy.

MATERIAL and METHODS

Eighty-five patients with laryngeal carcinoma who were evaluated in this study were all diagnosed and treated at Dokuz Eylül University Hospital and had complete follow-up information. Patients were treated by partial or total laryngectomy and neck dissection, followed by adjuvant radiotherapy and close/involved margins or neck involved with multiple nodes or perinodal invasion patients received concomitant cisplatin-based chemotherapy.

Age, gender, tumor location, clinical tumor stage and lymph node stage, type of surgery, pathological tumor and lymph node stage and the number of metastatic lymph nodes, perinodal invasion, tumor grade, treatment scheme with radio/chemotherapy time and localization of loco/regional recurrence and distant metastasis were determined from the medical records of the patients. Second primary tumors, time of disease-related and all-cause death, and the date of the last follow-up time were recorded.

Histopathological evaluation

Hematoxylin and eosin (H&E) stained sections of the patients with SCC were re-evaluated and 5 digital images were taken from adjacent tumor areas including deepest invasive margin, from the regions with most abundant stromal component, for each case by a 3-CCD color video camera (Olympus DP70, Olympus Optical Co. Ltd., Tokyo, Japan), connected to a light microscope (Olympus BX51, Olympus Optical Co. Ltd., Tokyo, Japan) at an original magnification of x20 and they were stored at a personal computer, only excluding large necrotic regions.

Image Analysis

TSP was measured at the images mentioned above by a novel software tool developed in C++ with a Graphical User Interface for the Windows operating system Histopathological Image Atlas Editor (HIAE), allowing the pathologists to mark square blocks of the target area in two types by left or right clicking by mouse and automatically calculating the proportion of the marked areas. The two types were tumor or stroma in this study. The area of square regions, delineated by the software program automatically, which could be marked by the pathologist, was 205.6 μm^2. The final result for each case was calculated by the mean of the TSP's of five images which were obtained by examining a 3.451 mm^2 area which corresponds to 16.785 square blocks in total. The selection of each case took about fifteen minutes. A screen snapshot of the HIAE is presented in Figure 1. In this figure, the main window of the HIAE displaying a histopathology slide under examination is presented. Individual square regions that were marked by the expert were merged and framed onto the image and also the coverage percentage of the markings was presented by a pie chart. In this case, the areas selected as red are the tumor and the yellow are stromal components.

Statistical analysis

The cases were grouped by TSP as low and high according to the mean of the TSP values of all the cases. Statistical analysis was conducted by Scientific Package for Social

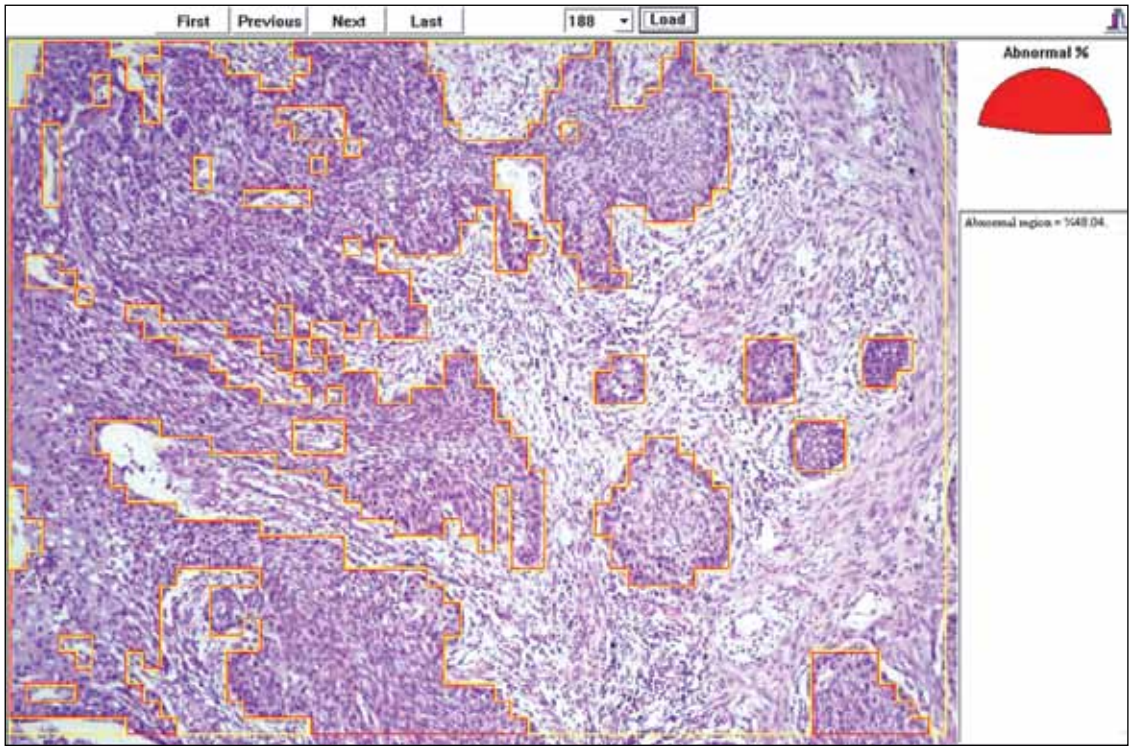

Figure 1: An image sample of selected tumor area with the software Histopathological Image Atlas Editor.

Sciences (SPSS 11). For comparison of these groups, nonparametric tests such as chi-Square were applied. The overall and disease free survival analysis was plotted by the Kaplan Meier method. For comparison of the survival of different groups, the log rank test was applied for unvaried analysis with 95% confidence interval and Cox-regression for multivariate analysis. Localization of tumor, clinical and pathological, tumor and lymph node stage, number of metastatic lymph nodes, perinodal invasion, and differentiation were evaluated as variables.

RESULTS

The study population consisted of 81 (95.3%) males and 4 (4.7%) females. The mean age of the patients was 57 years (range, 36–86 years). Eight (9.4%) patients underwent partial and 77 patients (90.6%) underwent total laryngectomy. Eighty-one patients had classical SCC and 4 (4.7%) were of the basaloid subtype. Some clinical and prognostic features are given in Table I. Thirty-five cases (41.2%) were supraglottic, 27 (31.8%) were glottic, 4(4.7%) were subglottic, and 19 (22.4%) were transglottic. Pathological T stages were as follows: T2 for 5 patients (5.9%), T3 for 41 patients (48.2%), and T4 for 39 patients (45.9%). Thirty-one patients (48.2%) had no lymph node metastasis (pN0), and the numbers of pN1, pN2, and pN3 cases were 18 (21.2%), 25 (29.4%), and 1 (1.2%) respectively. Twenty patients (23.5%) had perinodal invasion. All patients were considered for post-operative radiotherapy for the following reasons: pT stages 3 and 4, pN (+), patients with pT2 and a positive surgical margin, and/or subglottic extension. All patients received adjuvant radiotherapy, and received concomitant chemotherapy according to the criteria described above.

Median follow up was 48 months (range 3-194). The mean TSP was 48.63$\pm$18.18. There was no difference for TSP when sex, tumor location, tumor grade, pathological and clinical tumor and lymph node stage, and perinodal invasion were considered (p=0.36, p=0.21,p=0.24, p=0.41, p=0.40, p=0.83, respectively). Although statistically insignificant, the mean TSP was the lowest for subglottic carcinomas compared with supraglottic, transglottic and glottic cases (37.46$\pm$12.49, 52.41$\pm$37.47, 50.86$\pm$19.84 and 44.56$\pm$16.91, respectively). Also TSP was lowest in cases with perinodal invasion compared with cases with metastasis but no perinodal invasion and highest in cases without lymph node metastasis (44.72$\pm$20.23, 47.77$\pm$17.37, 50.05$\pm$17.34, respectively). The mean value of TSP was higher in basaloid subtype compared with the classical SCC cases (53.76±14.70 and 48.63±18.38, respectively).

The overall and disease free survival analysis did not reveal significance with log rank for sex, tumor location, tumor grade, clinical tumor and pathological lymph node stage, and perinodal invasion as well as TSP, but significance for clinical lymph node stage (p=0.036), pathological T stage

(p=0.01), pathological stage (p=0.016) and number of metastatic lymph nodes (p=0.00) (Figure 2, Table II). Only pathological stage was an independent factor with cox-regression for overall survival with 95% confidence interval (p=0.008, hazard ratio=1.003; lower: 1.001, upper: 1.005) among the latter four features with statistical significance.

DISCUSSION

Recent findings highlight the importance of TSP as a prognostic marker in some types of cancer (5-11). High proportion of stroma seems to be a poor prognostic marker (5-11).

Table I: Demographical and prognostic distributions of all cases according to proportion of tumor-stroma

			Proportion of tumor-stroma				
	All cases		High		Low		
	n	%	n	%	n	%	P value
Gender							0.36
Male	81	95.3	39	48	42	52	
Female	4	4.7	1	25	3	75	
Location							0.21
Glottic	27	31.8	9	22.5	18	40	
Supraglottic	35	41.2	19	47.5	16	35.6	
Subglottic	4	4.7	1	2.5	3	6.7	
Transglottic	19	22.4	11	27.5	8	17.8	
Grade							0.24
well	24	28.4	13	32.5	11	24.4	
moderate	42	49.4	20	50	22	48.9	
poor	19	22.4	7	17.5	12	26.7	
pT							0.41
pT1	0	0	0	0	0	0	
pT2	5	6	1	2.5	4	9	
pT3	41	48	21	52.5	20	44.4	
pT4	39	46	18	45	21	47	
pN							0.40
pN0	41	48.2	20	48.8	21	46.7	
pN1	18	21.2	11	27.5	7	15.6	
pN2a	4	4.7	1	2.5	3	6.7	
pN2b	16	18.8	5	12.5	11	24.4	
pN2c	5	5.9	3	7.5	2	4.4	
pN3	1	1.2	0	0	1	2.2	
Pericapsular invasion							0.83
Yes	20	23.5	9	22.5	11	24.4	
No	65	76.5	31	77.5	34	75.6	
Local Recurrence							0.90
Yes	4	5	2	5	2	4	
No	81	95	38	95	43	96	
Distant recurrence							0.82
Yes	12	14.1	6	15.0	6	13.3	
No	73	85.9	39	53.4	34	46.6	

Table II: Overall survival analysis with Log rank and "p" value. The standard error was calculated with 95% confidence interval

	3 year overall survival rate	P value	Mean	Std. Error	Lower bound	Upper bound
TSP		0.381				
< 48.63	43%		113	12	89	136
>48.63	50%		133	15	103	163
Gender		0.088				
Male	78%		124	9	105	143
Female	50%		38	13	11	65
Location		0.720				
Glottic	39%		113	13	87	139
Supraglottic	33%		111	15	81	141
Subglottic	50%		141	34	74	208
Transglottic	71%		107	13	81	134
Clinical tumor stage		0.748				
T2	73%		129	23	82	175
T3	84%		126	12	102	150
T4	82%		89	11	67	111
Clinical N stage		0.008				
N0	75%		131	10	110	152
N1	51%		38	8	21	55
N2	80%		97	23	52	143
Clinical stage		0.187				
Stage II	78%		109	16	76	142
Stage III	85%		137	13	110	163
Stage IV	82%		99	14	70	128
Pathological tumor stage		0.010				
T2	100%		136	0.7	134	137
T3	81%		111	13	85	137
T4	85%		128	14	100	157
Pathological N stage		0.370				
N0	42%		118	8	101	136
N1	87%		121	22	77	165
N2	74%		107	17	74	141
Pathological stage		0.016				
Stage II	100%		136	0.7	134	137
Stage III	87%		115	15	85	145
Stage IV	82%		122	12	98	146
Perinodal invasion		0.534				
+	82%		102	16	69	136
-	84%		124	11	102	146
Grade		0.434				
I	86%		139	15	109	169
II	88%		102	11	80	124
III	66%		116	21	73	159

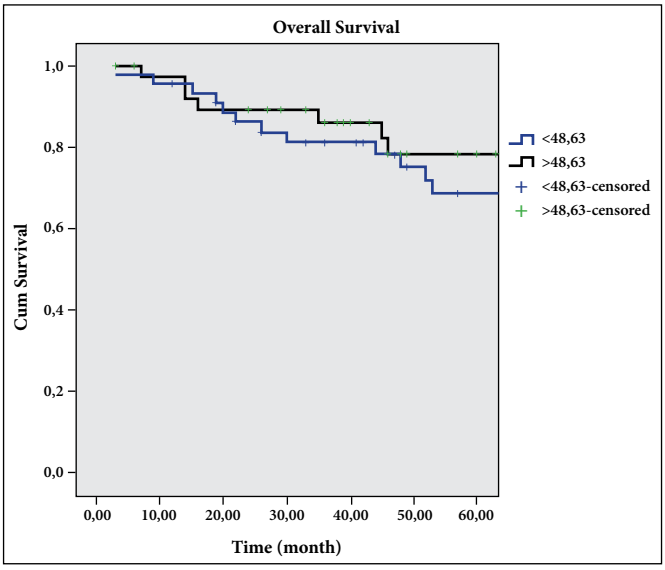

Figure 2: Graphic for overall survival.

The importance of tumor-stroma interaction was recognized a long time ago. Stephan Paget, a surgeon and son of Sir James Paget who is recognized as the founder of scientific medical pathology, proposed the "seed and soil" hypothesis in 1889. He concluded that "the best work in pathology of cancer is done by who are studying the nature of the seed (the cancer cell); the observations of the soil (the secondary organ) may also be useful" (26). During the last three decades there is increasing evidence that support the role of tumor stroma in tumor invasion, metastasis, progression and prognosis.

In 1993 Dingemans et al. presented the infiltrative phenotype of colon carcinoma cells when transplanted into granulation tissue while they formed encapsulated and well differentiated tumors at undisturbed subcutaneous tumors in an experimental model (27). This study presented direct evidence for the importance of tumor stroma in the prognostic phenotypical features of tumor growth like the infiltrative pattern. Many other factors about tumor stroma have also been described. The tumor microenvironment consists of extracellular matrix, immune and inflammatory cells, blood vessel cells and myofibroblasts (28). The myofibroblasts, fibroblastic cells adjacent to cancer cell nests expressing α-smooth muscle actin, seem to be very important contributors of the tumor stroma through the secretion of matrix metalloproteinases (MMP) and their inhibitors (TIMP), cytokines and chemokines along with the neoplastic cells (29). Degradation of the matrix and the MMPs are crucial for invasion and metastasis (30). It is suggested that platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) expression induce myofibroblastic cell proliferation (31,32). Myofibroblasts are derived either from the bone-marrow or the multiple resident precursors; the endothelial cells, smooth muscle cells, adipocytes and stellate cells (29).

Recently tumor stromal cells have been shown to participate in the Warburg effect in a reverse pattern, through the stimulation of the hydrogen peroxide secretion by the tumor cells inducing pseudohypoxia in the tumor microenviroment. The stromal cells then produce and present L-lactate and ketone as well as nucleotides, fatty acids and amino acids, such as glutamine to the tumor cells as a result of aerobic glycolysis and mitophagy (33,34). All these above mentioned data support the stroma as an important predictor of tumor behavior.

The association of hypoxia, fibrosis and poor prognosis has been recognized in various tumors for some time (35). The production of more growth factors by more stromal cells and the encapsulation of the tumor cells by fibrosis not allowing the penetration of the immune response were proposed to explain this association along with the mechanisms described above (32,33,37,38). Depending upon all these findings, the morphological quality and quantity of tumor stroma is evaluated in different tumors as prognostic markers. The properties of the stroma are described as a prognostic marker in rectal cancer by Ueno et al who proposed mature, intermediate and immature categories (12). They identified more tumor budding, which is also a poor prognostic factor in colorectal carcinomas as well as poor prognosis in cases with immature stroma (12,38,39).

The quantity of stroma or stromal components was also described as prognostic features. Sis et al. reported desmoplasia measured by computer assisted image analysis in van Gieson stained sections as a poor prognostic factor in colorectal carcinoma (13). Mesker et al. proposed carcinoma stroma ratio as a prognostic marker in colorectal carcinomas (6). They estimated the TSP in tumor sections and the scored lowest percentage was considered as decisive. In this study, 50% was accepted as the cutoff point for dichotomatous categorization, and they identified tumor percentage as an independent prognostic factor in Cox regression analysis. This was followed by the study in oesophageal adenocarcinoma by Courrech Staal et al., the same method was applied but in this study "tumor-stroma ratio" terminology was used instead of percentage and the tumor stroma ratio was identified as a highly significant prognostic factor (9). Moorman et al (10) and de Kruijf et al. (8) presented the prognostic value of tumor-stroma ratio in triple negative breast adenocarcinomas with methods

similar to Mesker et al (6). The importance of the tumor stroma ratio is also validated by the morphometric study by West et al. in colorectal adenocarcinomas (7).

The above mentioned findings are in favor of TSP as a prognostic factor for adenocarcinomas, but there is no information about SCC other than articles about desmoplasia. The association of desmoplastic response and unfavorable prognostic histopathological findings and recurrences were described for skin SCC (14-17). de Diego SJI (40) could not detect any relationship between desmoplasia with local recurrence in laryngeal and hypopharyngeal carcinomas, but Prim et al. (41) presented the desmoplasia at metastatic lymph nodes as a prognostic marker. Zidar et al. reported well differentiated SCC morphology and desmoplasia association in laryngeal SCC but they did not present prognostic data (42). Tumor budding, a poor prognostic finding related to the type of desmoplasia in colorectal carcinomas, is also described as a prognostic marker for laryngeal SCC (12,25). Based on the lack of information about TSP in laryngeal carcinomas and the above mentioned findings, we investigated if there was any relationship between TSP and histopathological findings and survival in laryngeal SCC.

Two methods were applied in the previous series in order to determine TSP. de Kruijf et al. (8), and Courrech Staal et al. (9) applied the methodology described by Mesker WE et al. (6), and they used semiquantitative measurements depending upon the estimation of the pathologists by visual inspection, while West et al. (7) performed point counting with a grid of 300 points and counted tumor-stroma proportion in a 9 mm^2 tumor region. While the former authors had selected the deepest invasive border of the neoplastic lesions at the most stroma rich regions, West et al (7) preferred selecting areas from the luminal surface with greatest tumor cell density. These methodological differences highlight the requirement for a standard method for these measurements. In our series, we evaluated the cases as described by Mesker et al, including the invasive border but also we developed and used software allowing precise measurement in a short time. The cut point was 50% in previous series as described originally by West et al. and we used the mean value of 48.63 which was pretty close to this value (7).

The prognostic factors in laryngeal carcinomas include tumor location, tumor type, pathological T and N stage, grade, lymphovascular invasion, perineural invasion and perinodal invasion (20-23,41,42). In this series, some of the robust prognostic markers like tumor location, grade, tumor and lymph node stage were not identified as prognostic factors as well as TSP and only pathological stage was an independent prognostic factor for overall survival. Although there are conflicting results about SCC of the larynx in terms of desmoplasia, histopathological prognostic features and prognosis, considering the results in this series it is hard to conclude that the TSP is not a prognostic marker in laryngeal carcinomas (40-42). Although statistically insignificant, the lower TSP identified associated with lymph node metastasis and perinodal invasion as well as subglottic localization, which are poor prognostic factors in laryngeal carcinomas, suggests that further evaluation may provide significant results.

It seems TSP is an important factor that needs further evaluation in SCC as well as adenocarcinomas. Although we could not find statistical significance in this series for TSP, the quantitative method described in this series might be helpful for determining TSP in other series.

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