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Expression of CD105 but not of E-cadherin is associated with malignancy recurrence and disease-free interval in laryngeal cancer in men

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Abstract

Introduction. In this study we analyzed CD105 (endoglin) and E-cadherin expression in laryngeal squamous cell carcinoma (LSCC) to evaluate their clinicopathologic significance.

Material and methods. Expression of CD105 and E-cadherin was examined immunohistochemically using paraffin-embedded archival tissues of 72 (35 glottic and 37 supraglottic) previously untreated LSCC male patients. The mean value of the positively-stained microvessels for CD105 counted in four hot spots for each case was used as the final intratumoralmicrovessel density (MVD). A staining score of E-cadherin was calculated based on the percentage of cells stained (0–100%).

Results. MVD was significantly higher in patients with advanced TNM stage (P = 0.004) and younger than 65 (P = 0.008). Nodal metastases were more frequent in the cases with low E-cadherin expression (P = 0.000). Tumor recurrence was associated with advanced TNM stage (P = 0.035) and high MVD (P = 0.002). A high MVD was an independent predictor of malignancy recurrence (P = 0.021). The log-rank test showed a significant difference in the disease-free interval in patients stratified according to the MVD value (P = 0.016). Spearman's rank correlation test did not show a significant correlation between E-cadherin and CD105 expression.

Conclusions. CD105-assessed MVD and expression of E-cadherin are promising prognostic factors for the outcome of patients with LSCC. Increased expression of CD105 could help predict patients with an increased risk of developing loco-regional recurrence after surgical treatment. Decreased E-cadherin expression is a potential predictor of lymph node metastases. *(Folia Histochemica et Cytobiologica 2023, Vol. 61, No. 3, 183–192)*

Keywords: laryngeal carcinoma; CD105; angiogenesis; E-cadherin; MVD; immunohistochemistry

Introduction

Laryngeal squamous cell carcinoma (LSCC) accounts for 0.89% of all cancers diagnosed annually worldwide; the male-to-female incidence ratio is 5:1 [1]. Despite recent improvements in diagnostic, surgical and medical approaches, there has been no improvement in the 5-year survival rate in the last 40 years [2]. The presence of cervical lymph node metastases and local recurrence are still the most important adverse prognostic factors for LSCC. Tumor, node, and metastasis (TNM) classification, primary tumor site, and histopathological grading do not consider the specific tumor's biological characteristics. Therefore, there is a need for improvement in risk assessment, and identifying novel biomarkers could assist in the diagnosis, prognosis, and therapy of LSCC.

A recent definition of a prognostic factor describes it as "a situation or condition, or a patient's characteristic, that can be used to estimate the chance of

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recovery from a disease or the chance of the disease recurring" [3]. The abundant data regarding prognostic factors in LSCC are available in the literature [4–11]. Bradford *et al*. reviewed various prognostic factors in patients diagnosed with LSCC, grouped into the host (age, gender, nutritional status, performance status, and immunological response), tumor (tumor site, TNM stage, histological grade, second primary cancer), and treatment factors [2].

Tumor angiogenesis is the formation of new peri- and intratumor blood vessels. After an initial avascular phase of the development of solid tumors, angiogenesis is a crucial process to its growth and metastasis in a later vascular stage. The assessment of intratumoralmicrovessel density (MVD) is a well-established method to assess the extent of angiogenesis determined by counting the number of blood vessels or the intensity of their marker/s staining in a given area [12]. Tumor angiogenesis is measured using antibodies against endothelial cell markers such as vascular endothelial growth factor (VEGF), Factor VIII, CD31, CD34, and CD105 (endoglin) [13–16]. Endoglin, a cell membrane glycoprotein of 180 kDa, is a coreceptor of pleiotropic cytokine transforming growth factor β (TGF-β) that modulates angiogenesis by regulating different cellular functions. The CD105 gene is located on chromosome 9q34 [13]. Endoglin is a hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells [17]. It has shown a greater affinity for tumor vasculature than pan-endothelial markers such as CD34, CD31, and VEGF [13–16, 18]. Several studies have confirmed that quantification of CD105 assessed MVD is a powerful prognostic marker in LSCC [19–23].

Epithelial-mesenchymal transition (EMT) is associated with tumor progression, promoting cancer cell invasion and metastasis [24]. A critical molecular process during EMT is the "cadherin switching" in which the regular expression of epithelial cadherin (E-cadherin) is down-regulated and associated with enhanced migratory and invasive traits [25]. Cadherins are a group of adhesion molecules that mediate cell-cell interactions in the presence of calcium ions. There are many different classes of cadherins including E-cadherins, placental cadherins (P-cadherins), neural cadherins (N-cadherins) and liver cell adhesion molecule (L-CAM) [25]. E-cadherin is essential for the maintenance of epithelial integrity and plays important role in the formation of tissues during gastrulation, neurulation and organogenesis [25–28]. E-cadherin is connected with the cytoskeleton through β-catenin and maintains cell-cell adhesion. The down-regulation of E-cadherin is associated with the release of β-catenin, activating Wingless (Wnt) signaling, while the abnormal expression of N-cadherin, vimentin, and fibronectin is increased [25, 29]. E-cadherin expression is reduced or absent in several carcinomas, including LSCC, and usually, it is associated with lymphatic invasion and tumor metastasis of the disease [25, 30–32]. However, several other studies have failed to show a relationship between E-cadherin expression and these clinicopathological features in LSCC [33–35].

A large epidemiological study examining 17125 patients showed that the female sex is an independent prognostic factor for increased probability of survival in patients with LSCC [36]. Data also support that sex is an important factor in the pathogenesis and prognosis of head and neck squamous cell cancers [37, 38].

The present study aimed to assess the correlation of immunohistochemical expression of CD105 and E-cadherin in male patients with LSCC and determine its relationship with other clinicopathological parameters and clinical outcomes. To have a homogenous group of patients and exclude the influence of gender, we included only men in the study.

Material and methods

Seventy-two male patients with primary LSCC (35 glottic and 37 supraglottic) were included in this study. The clinical information, including sex, age, histologic grade, primary tumor (T) classification, nodal (N) status, TNM stage, and oncological outcome, were obtained retrospectively from clinical records at the Clinic for Otorhinolaryngology of the Clinical Center of Montenegro in Podgorica, Montenegro. The age of the patients ranged from 38 to 80 years (59.4 ± 9.1, mean ± SD). The tumor stages were T1 to T4, N0-N2, and M0. Thirty-nine patients had early cancer (stage I or II), and thirty-three had advanced cancer (stage III or IV) determined according to the 8th Edition of TNM Head and Neck Cancer Classification [39]. All patients had undergone primary partial (52 cases) or total laryngectomy (20 cases) with unilateral or bilateral cervical lymph node dissection. None of the patients was found to have had distant metastases during surgery. Patients with second primaries or who had received primary radiotherapy and/or chemotherapy were not considered. All patients had undergone microlaryngoscopy with laryngeal biopsy, upper aerodigestive tract endoscopy, neck and liver ultrasonography, head and neck computerized tomography (CT), and/or magnetic resonance imaging, and chest X-ray. The stage of disease was determined after the surgical resection of the tumor [39].

The clinical follow-up was adjustable to patients' characteristics and scheduled as follows: between 4 and 8 weeks in the first two years; every three months for year 3; every six months for years 4 and 5; and once a year thereafter. Neck ultrasonography and/or CT, chest X-ray and/or CT, liver ultrasonography, and total-body positron emission tomography were repeated if clinically indicated. The mean follow-up was 36.4 months, ranging

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from 6 to 60 months. A poor oncological outcome is defined as the recurrence of disease or occurrence of metastasis after treatment. Disease-free survival was calculated from the period of treatment completion until the date of tumor relapse. Fifteen of 72 patients (20.8%) developed loco-regional malignancy recurrence (5 local recurrences, 10 recurrences to neck lymph nodes). The clinicopathological characteristics of the selected patients are shown in Table 1.

Tissue processing and immunohistochemistry. We performed immunostaining on formalin-fixed, paraffin-embedded tissue sections using the EnVision System (DAKO Autostainer, model VL1, Dako, Glostrup, Denmark). Immunohistochemistry was carried out following the manufacturer's recommendations. All included samples originated from complete resection material. We selected the best section from each block showing central and peripheral areas of the tumor, avoiding areas with necrosis. The biopsy specimens were fixed in 10% phosphate-buffered

Table 1. Descriptive statistics of conventional clinical-pathological features of 72 patients and the correlation with CD105-assessed microvessel density (MVD) and E-cadherin expression

	N (%)	Mean MVD	± SD	p value	E-cadherin (%)	± SD	P value
All	72 (100%)	12.68	4.06		28.60	19.36	
Age (years)				0.008			0.361
≤ 65	53 (75.3%)	13.43	4.04		27.34	19.47	
> 65	19 (24.7%)	10.58	3.40		32.11	19.13	
Primary tumor site				0.151			0.771
Glottic	35 (48.6%)	11.97	3.82		29.29	20.22	
Supraglottic	37 (51.4%)	13.35	4.22		27.95	18.77	
T stadium				0.162			0.842
T1	18 (25.0%)	11.78	4.11		32.17	21.20	
T2	32 (44.4%)	12.44	3.63		26.00	17.81	
T3	18 (25.0%)	14.44	4.65		29.61	19.27	
T4	4 (5.6%)	10.75	2.63		28.75	28.09	
T stadium (early & advanced)				0.131			0.805
T1 and T2	50 (69.4%)	12.20	3.78		28.22	19.12	
T3 and T4	22 (30.6%)	13.77	4.55		29.45	20.33	
N stadium				0.161			0.073
N0	57 (79.2%)	12.25	4.12		31.54	20.44	
N1	8 (11.1%)	14.00	2.78		15.38	10.06	
N2	7 (9.7%)	14.71	4.31		19.71	3.59	
N0 & N+ category				0.076			0.000
N0	57 (79.2%)	12.25	4.12		31.54	20.44	
N+	15 (20.8%)	14.33	3.46		17.40	7.82	
Stage				0.038			0.754
Stage I	18 (25.0%)	11.78	4.11		32.17	21.20	
Stage II	21 (29.2%)	11.14	3.10		30.14	20.09	
Stage III	24 (33.3%)	14.25	4.25		26.33	18.29	
Stage IV	9 (12.5%)	13.89	4.19		23.89	18.06	
Stage — early & advanced				0.004			0.240
Stage I and II	39 (54.2%)	11.44	3.57		31.08	20.36	
Stage III and IV	33 (45.8%)	14.15	4.17		25.67	17.98	
Histological grade (HG)				0.763			0.331
HG I	32 (44.4%)	12.84	4.41		31.09	21.17	
HG II	40 (55.6%)	12.55	3.82		26.60	17.81	

Results are expressed as mean and standard deviation (SD).

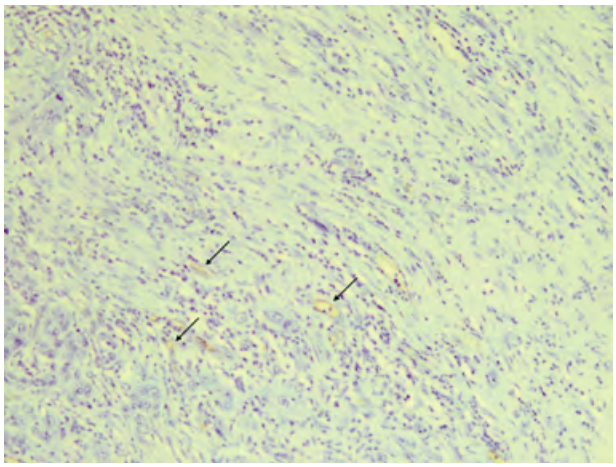

Figure 1. Low MVD in laryngeal squamous cell carcinoma as assessed by CD105 Ir. Arrows indicate CD105-Ir in endothelial cells of only a few blood vessels. IHC staining was performed as described in Methods. Magnification: 100×. Abbreviations: IHC — immunohistochemical; Ir — immunoreactivity; MVD — mean vessel density.

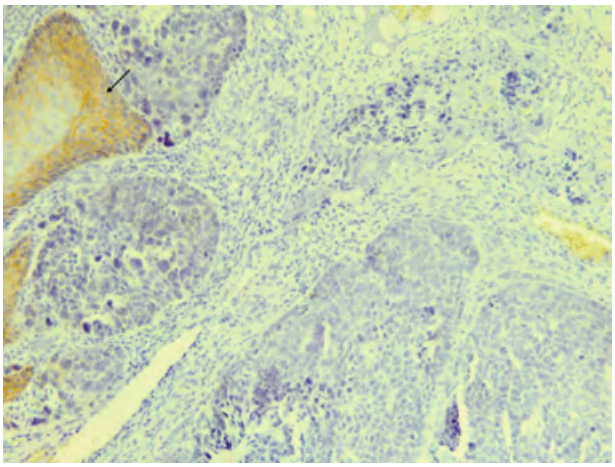

Figure 3. Low expression of E-cadherin in laryngeal squamous cell carcinoma. Arrow indicates membrane positivity in a small number of tumor cells. Immunohistochemical staining was performed as described in Methods. Magnification: 10×.

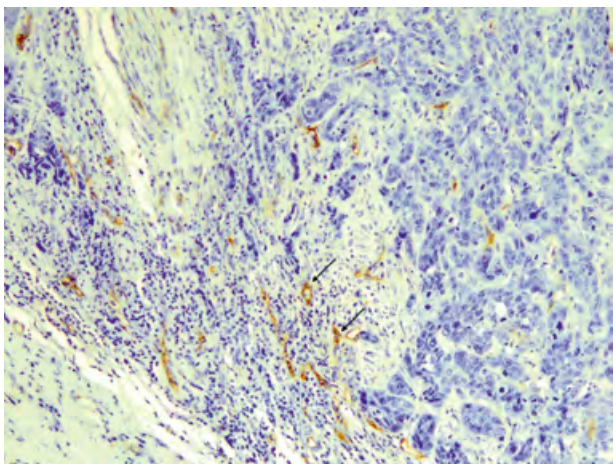

Figure 2. High MVD in laryngeal squamous cell carcinoma as assessed by CD105-Ir. Arrows indicate CD105-Ir in endothelial cells of many blood vessels. IHC staining was performed as described in Methods. Magnification: 100×. Abbreviations as in the legend to Fig. 1.

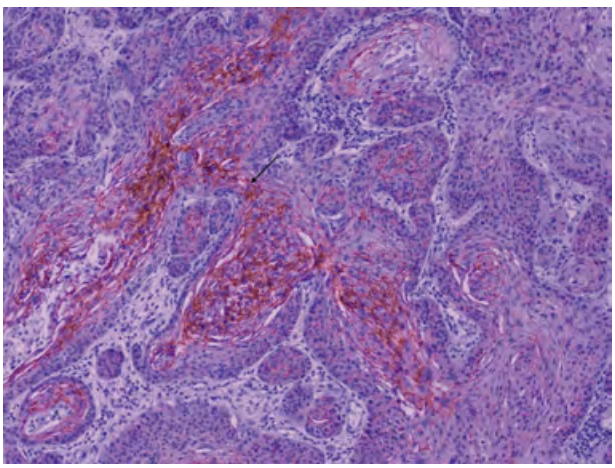

Figure 4. High expression of E-cadherin in laryngeal squamous cell carcinoma. Arrows indicate membraneous immunoreactivity in most tumor cells. Immunohistochemical staining was performed as described in Methods. Magnification: 10×.

formalin, processed for obtaining 4-μm-thick paraffin sections, and deparaffinized. The endogenous peroxidase's activity was blocked with a methanol solution containing 3% hydrogen peroxide for 10 min. Tissue sections were washed with TRIS--buffered saline and then incubated for 30 min with mouse monoclonal antibodies against E-cadherin (Clone NCH- 38 diluted 1:50, DAKO) and CD105 (Clone SN6h diluted 1:20, DAKO). All sections were subjected to a heat-induced antigen retrieval process (pH:8,0, 95°C). 3,3'-diaminobezidine (DAB, DAKO) was used as a chromogen for 10 min. The slides were then counterstained with hematoxylin.

A section of normal laryngeal mucosa was used as a positive control for E-cadherin (epithelial cells) and CD105 (endothelial cells) expression. The primary antibody was omitted for negative controls, and Tris-buffered saline was used for both markers.

Immunohistochemical evaluation. A pathologist without knowledge of the clinical data randomly evaluated the slides. Representative images of each staining are shown in Figs. 1–4. The intratumoral MVD quantification was performed under light microscopy following the method proposed by Weidner *et al.* [40]. The sections were scanned at 40× magnification to select four areas with the highest vascular density ("hot spots"). CD105- positive endothelial cells were counted using the 200× magnification. Any endoglin-stained single cell or cell cluster separated from the adjacent microvessels, tumor cells, or other

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connective tissue elements was considered a countable vessel. A visible vascular lumen was not required to count as a microvessel. The rounded mean value of the vessel count in four fields for each case was used as the final MVD value. A cut-off point identified by the receiver operating curve (ROC) was chosen to separate patients with high and low MVD.

For the immunostaining of E-cadherin, all stained cells were considered positive regardless of the intensity of the staining. The staining was predominantly membranous, with some cytoplasmatic staining. The immunohistochemical staining score of the E-cadherin expression was given based on the percentage of cells stained (0–100%) according to previously described criteria [12]. The ROC approach was applied to determine the cut-off value. The patients were classified as low expressers (E-cadherin expression below the cut-off value of the staining scores) and high expressers (E-cadherin expression above the cut-off value).

Ethical statement. The study was approved by the Ethics Committee of the Clinical Center of Montenegro (Approval number: 03/01/5068/1, date: 24.03.2022) and conducted following the Declaration of Helsinki. All patients preoperatively signed a consent form to disclose privacy in managing personal data for scientific purposes. Before undergoing surgery, all patients included in the study signed a detailed informed consent form. We did not need additional informed consent to use the specimens in this study because only archived material was used.

Statistical analysis. Descriptive statistics were expressed as frequencies and percentages for categorical variables and mean, standard deviation (SD), and range (minimum and maximum) for continuous variables. The correlation of CD105 and E-cadherin expression levels with clinicopathological features was tested using Kruskal-Wallis, Pearson's χ-square, or Fisher's exact tests. The associations between the expression of E-cadherin and CD105 and LSCC prognosis were analyzed using Kaplan-Meier survival analysis, and the logarithmic rank test was used in the univariate analysis. The ROC approach was applied to determine the analytically best-fitting cut-off points of the variables selected for the subsequent survival analysis. The relationship between E-cadherin and CD105 was performed using Spearman's rank correlation analysis. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used before statistical analysis to determine the data distribution. P values lower than 0.05 were considered statistically significant. All statistical analyses were conducted with SPSS 26.0 for Windows package (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA).

Results

Many CD105-stained microvessels were observed within tumor tissues. CD105- immunoreactivity (-Ir) was observed in the endothelial cell membrane and cytoplasm of only a few blood vessels (Fig. 1) or many blood vessels (Fig. 2). CD105-assessed-MVD varied among tumor samples from 5 to 22 (median 13, mean 12.68 ± 4.06). The cut-off value identified by ROC for survival analysis was 12.5 for CD105 expression (sensitivity 100%, specificity 42%). Using the calculated cut-off value, 39 (54.2%) tumors were classified in the "high MVD" group, and the rest, 33 (45.8%) tumors, consisted of the "low MVD" group. The correlation of the MVD with clinicopathologic parameters is summarized in Table 1. MVD was significantly higher in patients with advanced (III and IV) clinical stage (Mann–Whitney P = 0.004). The Kruskal-Wallis test showed a significant association between differences in CD105 expression and TNM stages (P = 0.038). Interestingly, CD105 expression was significantly higher in patients 65 and younger than in the elderly group (older than 65 years) (Mann–Whitney P = 0.008). CD105 expression showed a trend toward a significant association with the presence of lymph node metastasis at the time of diagnosis (P = 0.076). MVD did not depend on the primary tumor's location and differentiation grade.

E-cadherin showed membraneous immunoreactivity in a small percentage of tumor cells (Fig. 3) or many tumor cells (Fig. 4). E-cadherin expression varied among tissue samples from 2% to 70% (median 24%). The mean E-cadherin expression was 28.60% ± 19.36%. The cut-off value for E-cadherin expression identified by ROC was 15.5% (sensitivity 80%, specificity 67%). Using the calculated cut-off value, 23 patients (31.9%) were classified as low expressers and 49 (68.1%) as high expressers. Table 1 summarises the correlation between E-cadherin expression and clinicopathologic parameters. There was a significant difference in the E-cadherin expression between tumors with and without nodal metastases: nodal metastases were more frequent in the cases with low E-cadherin expression (Mann–Whitney P = 0.000, Kruskal-Wallis P = 0.073). Statistical analysis ruled out an association between E-cadherin expression and age, primary tumor's location, T-stage, TNM-stage, and pathological grading.

Table 2 presents the correlation of tumor local and regional recurrence with clinicopathologic parameters, MVD, and E-cadherin expression. Statistical analysis showed that tumor recurrence was associated with advanced stage (III and IV) grouping (P = 0.035) and high CD105 expression (P = 0.002). We found a trend toward a significant association between advanced T-stage (T3 and T4) and locoregional carcinoma recurrence (P = 0.056). However, no correlation was found between the locoregional recurrence of disease and age, primary tumor's location, lymph node metastases, and E-cadherin expression.

Table 2. CD105-assessed MVD, E-cadherin expression and conventional clinical-pathological features according to laryngeal squamous cell carcinoma recurrence

Variables	Number of patients	Loco-regional recurrence		
		No	Yes	P value
Age (years)				0.096
≤ 65	53	39	14	
> 65	19	18	1	
Primary tumor site				0.298
Glottic	35	30	5	
Supraglottic	37	27	10	
T classification				0.056
T1 and T2	50	43	7	
T3 and T4	22	14	8	
N status				0.499
N0	57	46	11	
N+	15	11	4	
TNM stage				0.035
I and II	39	35	4	
III and IV	33	22	11	
CD105 MVD				0.002
Low	33	32	1	
High	39	25	14	
E-cadherin expression				0.358
Low	23	20	3	
High	49	37	12	

MVD — microvessel density; low MVD < 12.5, high MVD > 12.5; low E-cadherin expression < 15.5%, high E-cadherin expression > 15.5%.

In the univariate logistic regression analysis considering age, primary tumor site, tumor extension, lymph node metastases, TNM clinical stage, CD105, and E-cadherin expression, an accuracy of 83% was achieved, with a sensitivity of 40% and a specificity of 95%. The results showed that a high CD105-assessed MVD was statistically significant (P = 0.007) and an independent predictor of malignancy recurrence (Table 3).

The log-rank test showed a significant difference in the disease-free interval (in months) in patients stratified according to CD105-assessed MVD (log-rank × 2 = 5.779, P = 0.016 (Fig. 5).

Spearman's rank correlation test did not show a significant correlation between E-cadherin and CD105 expression (rho = 0.022, P = 0.857).

Discussion

LSCC is a common cause of morbidity and mortality worldwide. Yet, the 5-year overall survival rate did not significantly improve despite the progress in the basic science knowledge, diagnosis, and treatment [2]. Much research has concentrated on identifying the potential prognostic markers of LSCC for a better understanding of its biological behavior.

Tumor angiogenesis is a complex multistep process crucial for tumor growth and metastasis. Quantification of tumor angiogenesis, intratumor MVD is a promising independent prognostic marker in various malignancies, such as esophageal cancer [16], colon cancer [41], breast cancer [42], renal cancer [43], head and neck cancer [44]. MVD has been previously studied using panendothelial markers such as CD34, CD31, and von Willebrand factor, yet CD105 antibodies have confirmed a greater specificity for tumor vasculature [13–16, 18].

In the present study we found that high CD-105-assessed MVD values were associated with the unfavorable advanced clinical stage (Stage III and IV). Also, in our study, high MVD was nearly significantly associated with nodal metastasis. These findings are consistent with those reported by Martone *et al.* [44] and Kyzas*et al*. [45]. Martone *et al.* considered 107 cases of LSCC among 127 patients (122 males and 5 females) of primary head and neck SCC [44]. The high MVD correlated with advanced T tumors, positive N metastasis, and advanced TNM stages.

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Table 3. Univariate Cox regression analysis results for laryngeal squamous cell carcinoma recurrence

	Wald test	P	OR	95.0% CI
Age (≤ 65 to > 65 years)	0.903	0.342	0.35	0.03–3.53
Primary tumor site (supraglottic-glottic)	1.204	0.273	0.33	0.04–2.41
T stage (T3, 4–T1, 2)	0.086	0.770	0.65	0.04–11.66
N stage (N+– N0)	0.838	0.360	4.02	0.20–79.36
TNM stage (S3, 4–S1, 2)	0.834	0.361	0.21	0.01–6.06
CD105 expression (high-low)	5.366	0.021	0.07	0.01–0.66
E-cadherin expression (low-high)	1.081	0.299	2.38	0.46–12.19

Low and high MDV and E-cadherin expression as described in legend to Table 2. Abbreviations: MVD — microvessel density, OR — odds ratio, CI — confidence interval.

Gu *et al.* concluded that CD105-assessed MVD in 30 LSCC was significantly related to increased tumor aggressiveness, high grade of differentiation, and advanced clinical stages [46].

Among the several families of adhesion molecules, a reduced/absent expression or abnormal location of E-cadherin has been suggested to play a role in nodal metastasis in several carcinomas including gastric [47], prostate [48], colon [49], and breast cancer [50]. Recent studies have shown that the expression of E-cadherin might be a useful marker to predict the risk for lymph node metastasis in LSCC and identify patients who need additional treatment. Franchi *et al*. examined 58 males and 2 females treated for LSCC and observed that low expression of E-cadherin in LSCC significantly correlated with the presence of occult nodal metastases [51]. Rodrigo *et al*. investigated the prognostic significance of E-cadherin expression in 101 males with primary LSCC of the supraglottic larynx [52]. They concluded that there was a significant correlation between decreased E-cadherin expression and the presence of nodal metastases. Akdeniz *et al*., in their cohort of 36 males and 2 females with LSCC, found a significant correlation between reduced E-cadherin expression and lymph node metastasis and poor differentiation of the tumor [53]. Ahmed *et al*., in a cohort of 75 patients (70 males and 5 females), reported a significant association between lower expression of E-cadherin and lymph node metastasis, advanced T-stage and TNM stage, and poor tumor differentiation [54]. The presented study found that E-cadherin expression independently predicted lymph node metastases. This result is consistent with some studies on LSCC [51–54], whereas others have failed to show this relationship [55–57].

The current study showed that tumor locoregional recurrence was associated with advanced TNM stage (III and IV), and high CD105 expression. The advanced T category was nearly significantly associated with

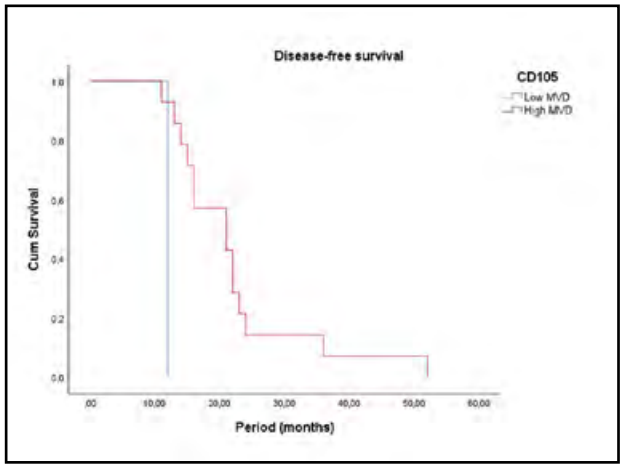

Figure 5. Kaplan-Meier curve for disease-free survival for 72 male patients with laryngeal squamous cell carcinoma according to the MVD determined by CD105 immunoreactivity. Abbreviation: MVD — microvessel density.

locoregional recurrence. However, no correlation was found between the locoregional recurrence of disease and age, primary tumor's location, N+ category, and E-cadherin expression. The univariate logistic regression analysis showed that a high CD105-assessed MVD was the only independent marker of locoregional tumor recurrence. We observed in the current study a significant association of CD105-assessed MVD with shorter disease-free survival and poor prognosis in patients with LSCC. Our observations support previous findings for the prognostic significance of CD105 in LSCC. Li *et al*. evaluated CD105-assessed MVD among 40 patients with primary LSCC. CD-105-assessed MVD was an independent indicator for predicting invasion, metastasis, and tumor recurrence [58]. The studies by Marioni *et al.* [59] and Martone *et al*. [44] concluded that a high CD105-assessed MVD was significantly related to tumor recurrence or death. The patients with a high MVD had signifi-

cantly shorter disease-free and overall survival [44]. Marioni *et al*. evaluated 62 cases with LSCC and found shorter disease-free intervals in patients with higher CD105 expression [19].

The correlation of angiogenesis with survival in LSCC makes it amenable to therapy with vascular targeting. The first approved antiangiogenic drugs, such as bevacizumab and sorafenib, are anti-VEGF agents. CD105 may represent an ideal target for antiangiogenic therapies for LSCC [60]. TRC105, IgG1 antibody that induces apoptosis in endoglin-positive tumor cells, has been recemtly tested in randomized phase II trials in glioblastoma and renal cell carcinoma patients [61, 62].

Although, as discussed above, the problem of endoglin expression in LSCC has been studied by many authors, we think that the main strength of the present study lies in the homogeneity of the patient population since all patients were male who underwent surgery by the same surgical team, only surgical specimens (not biopsies) were investigated, only squamous cell carcinomas of a specific structure (larynx) were considered, and clinical-radiological follow-up criteria were defined. A risk-based scoring at the time of diagnosis, which would incorporate various demographic parameters, histopathological features, and the combination of the expression of markers, would supply information on the biological behavior of tumors and help better stratify patients and select the most appropriate treatment.

The main weakness of our study concerns the retrospective single-center setting and the small sample size. Measuring the expression of examined markers in a small section of archival tissue may not always be appropriate due to the heterogeneity of tumors. Inter-observer variability in identifying and selecting the hot spots and cut-off points for separating tumors with high *vs.* low expression of specific markers can lead to discrepancies among different studies.

In summary, the results of our present study suggest that CD105-assessed MVD and expression of E-cadherin are promising prognostic factors for the outcome of male patients with LSCC. CD105-assessed MVD could help predict patients with an increased risk of developing laryngeal carcinoma loco-regional recurrence after treatment. Reduction of E-cadherin expression in LSCCs is an independent predictor of lymph node metastases, and its immunohistochemical determination might be useful in predicting the risk of nodal occult metastases at the time of diagnosis and identifying patients who may benefit from elective neck dissection.

Article information

Data availability statement

The datasets used and/ or analyzed in the present study are available from the corresponding author on request.

Ethics statement

The study was approved by the Ethics Committee of the Clinical Center of Montenegro (Approval number: 03/01/5068/1, date: 24.03.2022) and conducted following the Declaration of Helsinki.

Author contributions

Conceptualization — EZ; methodology — EZ, LV; formal analysis — EZ; investigation — EZ, LV; data curation — EZ, LV; writing — original draft preparation — EZ; writing — review and editing — EZ, LV.

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Conflict of interest

The authors declare that there is no conflict of interest.

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