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L1CAM expression is a prognostic biomarker for head and neck squamous cell carcinoma (HNSCC), a retrospective monocentric study

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Abstract

Beckground:

Head and Neck squamous cell carcinomas (HNSCC) are often diagnosed in late stages with regional lymph node metastasis. L1CAM (L1 cell adhesion molecule) is known as a significant prognostic marker in solid tumours such as endometrial and colon carcinoma and is often associated with invasion into the lymphovascular space and therefore poor prognosis. Here, we investigated L1CAM´s expression in HNSCC and determined its prognostic value.

Methods:

We analyzed L1CAM expression by immunohistochemistry in a retrospective cohort of 233 HNSCC patients of all head and neck locations. An immunoreactive score was developed and its association with clinic-pathological characteristics and its prognostic value evaluated for overall and relapse-free survival.

Results:

L1CAM-staining was observed at the cell membrane and was most intense at the invasion front. Tumor size, UICC stage and the risk factor alcohol consumption could be linked to high L1CAM expression. L1CAM-status was highly associated with unfavorable outcome, for relapse free (RFS) as well as overall survival (OS). In subgroup analyses, L1CAM status was equally important for all subgroups. Only for lymph node metastasis positive (N1) and higher UICC stage (3 + 4) subgroups, L1CAM status was not significant for outcome.

Conclusions:

The determination of L1CAM by immunohistochemistry could be a promising additional prognostic biomarker for the outcome of HNSCC for the major anatomical sites oral cavity, oropharynx, hypopharynx and larynx.

Introduction

In Germany, head neck squamous cell cancers (HNSCC) are frequent tumors with an occurrence rate of 5%, which ranks it the fourth most common malignant disease in males. In comparison, in women this percentage is lower with 1.8 per 100,000 [1]. Malignant tumors of the head neck area form a heterogeneous group due to occurrence at different anatomical sites. Most frequently, these are grouped into four categories - lips/oral cavity, oropharynx, hypopharynx and larynx [2]. Associated risk factors for the development of this squamous cell cancer are alcohol and tobacco consumption. HPV (human papilloma virus) infections of smooth tissue located in the head and neck area, especially with the high-risk HPV subtype 16, is another risk factor, even without or low alcohol and tobacco consumption in the patient’s history [3]. Therefore, in the past few years, squamous cell cancer of the oropharynx caused by

HPV infection in young white men gained more scientific attention and focus [4]. However, these patients show a better prognosis due to higher response rates to therapy than patients with HPV negative carcinomas.

Typical findings during the patient's first physical examination are non-healing ulcers in the oral cavity, neck swellings, globus sensation, dysphagia, otalgia, hoarseness or stridor [5]. More than half of the patients present themselves to a physician in later stages III and IV, which are already associated with larger tumors, local lymph node metastasis and/or distant metastasis.

Treatment options for HNSCC depend on anatomical accessibility, the patient's co-morbidities and preservation of function. Surgical resection (often with neck dissection) is performed most frequently for accessible tumors, sometimes with adjuvant radio-chemotherapy (RCT). Selected patients receive primary radiotherapy or radio-chemotherapy [6]. In molecular pathology, several biomarkers are routinely measured [7]. HPV-induced carcinomas overexpress of $p16^{inkA}$ as a result of HPV-driven oncogenesis [8]. Aberrant expression of p53, caused by mutations of the *TP53* gene, are strongly associated with resistance to chemotherapy [9]. An upregulation of programmed death-ligand 1 (PD-L1) leads to a decreased immune response due to the body's reduced ability to recognize tumor cells. Checkpoint inhibitors such as nivolumab or prembrolizumab are now used as targeted therapy to restore the body's immune functions [8]. High PD-L1 expression correlates with metastasis and poor prognosis, especially in oral squamous cell cancer [10].

The glycoprotein L1CAM (L1 cell adhesion molecule) is physiologically expressed in neuronal tissue. Furthermore, L1CAM was naturally found in human tongue, parotid gland, different segments of the gastrointestinal tract and the kidney in fetal tissue [11]. The molecule consists of an intracellular cytoplasmic structure, a transmembrane domain and an extracellular part with a fibronectin type 3 connected to an immunoglobulin-like domain. Thus, L1CAM is a member of the immunoglobulin superfamily [12]. Additional forms exist, which are not membrane-bound. These various forms are derived from the membrane-bound L1CAM by proteolytic cleavage by ADAM (a disintegrin and metalloproteinase) proteases [13]. In neuronal tissue, L1CAM regulates the development and growth of neuronal structures and their differentiation, myelinization, cell adhesion, as well as cell migration [13]. In cancer, L1CAM overexpression is associated with lymphovascular space invasion (LVSI) and the tendency for larger, infiltrating tumors as well as metastasis, resulting in worse outcome. E.g. in endometrial carcinomas, L1CAM is therefore suggested as a pre-operative diagnostic marker according to German guidelines since 2022 [14]. Even though endometrial carcinomas build a different malignant entity, they share two similarities with HNSCC. Both entities show an early manifestation of locoregional lymph node metastasis and an adverse outcome associated with L1CAM expression [15, 16]. For this reason, we aimed here at examining the impact of L1CAM expression in HNSCC and whether it is a suitable diagnostic marker for consecutive therapy determination.

Materials and methods

Tissue samples and clinicopathological characteristics

232 patients treated for HNSCC at the Department of Otorhinolaryngology, head and neck surgery at the Brandenburg University Hospital from 2010 to 2020 were included in this retrospective study. Patients younger than 18 Years and older than 91 years were excluded. For one patient, there was not sufficient material for staining available. This study was approved by our local ethics commission (MHB-Fontane file number: E-01-20211028), and all procedures were conducted in accordance with the guidelines for research involving human subjects, especially the Declaration of Helsinki.

All carcinomas were staged in accordance with the 8th edition of the TNM classification by the AJCC (American Joint Committee on Cancer) Staging Manual, section head and neck cancer. Additionally, subclassifications of the four different anatomical sites were taken into account [17]. Finally, tumor stages were determined by using the UICC (Union Internationale Contre le Cancer) classification. Cases of CUP (cancer of unknown origin) were excluded.

Clinicopathological characteristics, including age, sex, risk factors, TNM stages, grading, anatomical site/localization and treatment, were collected from the hospital's clinical data system (Medico, CGM Clinical Europe GmbH, Koblenz, Germany). Tobacco- and alcohol use were extracted from the patient's history without further definition of pack years or alcohol volume. Further data on patients' outcomes were obtained from the cancer registry (Krebsregister Berlin-Brandenburg). These data are summarized in Table 1.

Table 1
Patient Characteristics and L1CAM Expression Clinico-pathological parameters were correlated with the L1CAM Status by cross-tabulation and Fisher´s exact test (2x2 matrices), X^2-test, and ordinal-to-ordinal correlation.

Parameter	**number**	**L1CAM high (%)**	**p-value (L1CAM)**
All cases	232	112 (47.9)	
Sex			0.617
Male	188	89 (47.3)	
Female	44	23 (52.3)	
Age			0.211 (correlation)
30–40	3	3 (100)	
41–50	15	8 (53.3)	
51–60	77	42 (54.5)	
61–70	72	28 (38.8)	
71–80	50	22 (44.0)	
81–90	14	8 (57.1)	
91-	1	1 (100)	
> 70	65	31 (47.7)	1.000
smoker			0.855
No	35	16 (45.7)	
Yes	196	96 (48.9)	
Alcohol consumption			0.005
No	103	39 (37.9)	
Yes	128	73 (57.0)	
Nodal state			0.048 (Pearson X^2)
0	84	34 (40.5)	
1	28	12 (42.9)	
2	8	3 (37.5)	
2a	5	3 (60.0)	
2b	43	19 (44.2)	

Parameter	number	L1CAM high (%)	p-value (L1CAM)
2c	35	21 (60.0)	
3	9	3 (33.3)	
3a	2	2 (100)	
3b	2	2 (100)	
N0	83	33 (39.8)	0.206
N1	129	63 (48.8)	
T			0.025 (correlation)
1 (incl. 1a, b)	61	22 (36.1)	
2 (incl. 2a)	60	28 (46.7)	
3	37	18 (48.7)	
4 (incl. 4a, b)	60	34 (56.7)	
T ≤ 2	121	50 (41.3)	0.075
T > 2	96	52 (54.2)	
M			0.553
0	207	94 (45.4)	
1	12	7 (58.3)	
Grading			0.554 (correlation)
1	12	4 (33.3)	
2	125	63 (50.4)	
3	82	41 (50.0)	
4	1	1 (100)	
TNM8 (1 + 2)	79	27 (34.2)	0.037
TNM8 (3 + 4)	110	55 (50.0)	
Histology			0.161
Carcinoma in situ	4	0 (0)	
SCC not further specified	17	10 (58.8)	
SCC HPV+	59	26 (44.0)	
SCC HPV -	151	76 (50.3)	

Parameter	number	L1CAM high (%)	p-value (L1CAM)
Localisation			0.330
Base of tongue	29	15 (51.7)	
Tongue	5	3 (60.0)	
Mouth base	15	6 (40.0)	
Palate	10	5 (50.0)	
Tonsilla	47	20 (42.5)	
Oropharynx	23	15 (65.2)	
Sinus piriformis	10	6 (60.0)	
Hypopharynx	18	12 (66.6)	
Larynx	75	30 (40.0)	
P16			0.37
negative	157	78	
positive	62	26	
Ki67	105	47	0.27
< 50%	66	36	
≥ 50%			
Treatments			
RCT (primary)			
No	166	76	0.24
yes	62	34	
Radiatio (primary)			
No	205	98	0.83
yes	23	12	
Surgery			
No	102	57	0.035
yes	127	53	
RCT (adjuvant)			
No	190	90	0.60

Parameter	number	L1CAM high (%)	p-value (L1CAM)
yes	38	20	
Chemotherapy			
No	125	55	0.183
yes	103	55	
Radiatio (adjuvant)			
No	203	99	0.83
yes	24	11	
RCT with adjusted Platin dose			
No	207	102	0.41
yes	14	5	
Radiatio (palliative)			
No	208	96	0.059
Yes	20	14	
Percutaneous Endoscopic Gastrostomy (PEG)			
No	89	40	0.42
Yes	136	69	

OS was defined as the time from diagnosis to death of any reason. RFS was defined as the time from diagnosis to relapse, progression or metastasis. Follow-up ranged from 0.6 to 581.9 months (average 176.3 ± 152.3 months).

Immunohistochemistry and evaluation

Surgically removed, formalin-fixed paraffin-embedded tissue samples (FFPE) of patients were collected from the archive of the Brandenburg Institute of Pathology and thoroughly re-examined by a certified pathologist (TK). All specimens were further processed by the staff of the institute of pathology using standardized laboratory protocols. 4 µm-thick sections were prepared on positively charged slides (Flex, Agilent, Waldbronn, Germany). The staining procedure was performed on a BOND-III immunostainer (Leica Biosystems, Wetzlar, Germany). Antigen retrieval for L1CAM was performed using a citrate-based buffer (ER1, Leica Biosystems) at 100°C for 10 minutes. Endogenous peroxidases were inactivated using H_2O_2 (3%) before the application of the primary antibody by incubation at room temperature for 13 minutes. Monoclonal rabbit anti-L1CAM, clone BSR3, RTU (Vitro Master Diagnostica, Granada, Spain) obtained from Leica Biosystems (Wetzlar, Germany) was used as primary antibody. The detection was

performed using a two-step HRP/DAB system with subsequent counterstaining, using the Bond Polymer Refine Detection Kit (Leica Biosystems, Wetzlar, Germany).

Evaluation of the staining results was carried out together with an experienced pathologist (LM + TK). Only vital parts of the tumor were taken into account, and only membranous L1CAM staining was scored. The staining intensity was categorized into 0 (none), 1 (low), 2 (moderate) and 3 (high). Representative examples are shown in Fig. 1. Also, the percentage of positive L1CAM tumor cells (intensity 1 and higher) was estimated. Due to heterogeneous staining patterns, we scored cases as positive when at least one percent of tumor cells were L1CAM positive withan intensity of 1 and higher.

Histological structures, which physiologically express L1CAM, such as nerves, lymph follicles and blood vessels, were not considered. In some samples, an invasion front was seen as a border-like structure (Fig. 1).

Statistical analysis

Statistical analysis was implemented using IBM SPSS Statistics for Windows (Version 29.0, IBM Corp., Armonk, NY). Clinicopathological parameters were correlated to the L1CAM-status by cross-tabulation and Fisher's exact test, if not indicated otherwise. Survival analysis was performed by using the Kaplan-Meier algorithm with log-rank testing for between-groups as well as univariable and multivariable Cox regression. The inclusion threshold for the multivariable Cox regression was set at $p = 0.1$, the exclusion at $p = 0.2$. Parameters were included in conditional forward mode. Statistical significance was commonly set at $p < 0.05$, whereas p-values < 0.1 were interpreted as a trend.

Results

Cohort characteristics and L1CAM expression

This retrospective cohort consists of 232 HNSCC cases of all locations of head and neck. Clinico-pathological characteristics are summarized in Table 1. We included 188 (80.7%) male and 44 (18.9%) female patients, ranging from 30 to 91 years of age, with a median of 62 years. Consumption of tobacco was reported for 196 cases (84.1%) and alcohol for 128 (54.9%) cases (Table 1). 130 cases (55.6%) showed lymph node metastasis, 124 (53.4%) TNM stage III and IV. Only 62 of 219 reported cases were p16-positive (28.3%).

Membranous L1CAM expression (Fig. 1) was detected by immunohistochemistry and scored based on staining intensity higher than 1 in more than 1% of tumor cells. Almost half of the tumors were positive (112, 47.9%). Especially, the tumor invasion front was associated with intense staining (Fig. 1).

Positive L1CAM-status correlated with alcohol consumption and higher UICC-stage. In contrast, this was not the case for smoking, age, lymph node- and distant metastasis, grading, p16- or ki67-status. L1CAM-

positive tumors were less frequently removed by surgery and more often subjected to palliative radiotherapy.

The tumor locations, larynx, tonsil, and mouth-base had the lowest percentage of L1CAM-positive tumors, whereas hypopharynx, Sinus piriformis and oropharynx showed a high frequency of L1CAM positive tumors (Table 1). Statistically, larynx differed from the hypopharynx and oropharynx and there was a statistical trend for tonsil versus oropharynx and hypopharynx (Kruskal-Wallis Test).

Survival analysis and L1CAM expression

Survival analysis was performed using the Kaplan-Meier algorithm and Cox regression analysis for overall (OS) and relapse-free survival (RFS) for important clinico-pathological markers and L1CAM status.

Relapse-free survival (RFS) by Kaplan-Meier algorithm

Information on relapses was obtained for 215 cases. Relapses occurred in 62 cases (29.0%), whereas for 153 (71.0%), no relapse was reported. Due to the low number of reported relapses, the significance was generally lower than for OS.

In Kaplan-Meier survival analysis, there was no significant difference between males and females (p = 0.613). Relapses occurred more frequently in older patients (≥ 70 years, p = 0.006). There was no significant correlation with alcohol and tobacco abuse. The p16-status had a positive impact on RFS, but this was not significant (p = 0.187). N-status was associated with unfavorable RFS (p = 0.048). Tumor size (T1 + T2 vs. T3 + T4) correlated with RFS, but this was not significant (p = 0.179). UICC stages I and II compared to III and IV exhibited a statistical trend for better RFS (p = 0.096). Although grading was not significant for RFS, G1 had the best RFS, whereas G2 and G3 both showed comparable worse survival curves. Comparing the anatomical sites, we found no significant difference (p = 0.170) for RFS. However, survival curves suggest a more favorable outcome for oropharynx carcinoma (n = 22) and worse RFS for Sinus piriformis (n = 10). When analyzing the localizations summarized into the 4 common groups (lips/oral cavity, oropharynx, hypopharynx and larynx), log-rank p was 0.663. Results for RFS and OS are summarized in Table 2

Table 2
Survival analysis by Kaplan-Meier algorithm. For RFS, a calculation of a median survival was not possible for most parameters and is therefore omitted.

Parameter	Median survival (OS) [weeks]	95% CI (OS)	Log-rank p (OS)	Log-rank p (RFS)
All cases	126.3	78.9–173.7		
Age < 70	195.9	111.73–280.0	< 0.001	< 0.001
Age ≥ 70	78.1	55.5–100.8		
Male	223.6	54.0–393.1	0.561	0.613
Female	121.6	74.5–168.7		
Non-smoker	244.3	48.6–440.0	0.106	0.651
smoker	125.1	78.0–172.6		
No alcohol	195.9	111.0–280.9	0.017	0.899
alcohol	99.4	65.4–133.5		
N0	272.7	135.4–410.0	0.003	0.048
N1	105.7	65.7–145.7		
T ≤ 2 (T1 + T2)	244.9	154.1–335.6	< 0.001	0.179
T > 2 (T3 + T4)	73.7	41.8–106.0		
M0	170.0	106.2–230.8	0.012	< 0.001
M1	59.3	38.7–79.9		
G1	351.9	0.0–730.7	0.183	0.134
G2 + G3 + G4	125.1	78.3–172.0		
UICC 1 + 2	351.9	228.5–475.2	< 0.001	0.096
UICC 3 + 4	105.7	62.1–149.3		
Anatomical site			0.003	0.170
base of tongue	187.9	1.1–374.6		
tongue	50.6	38.9–62.2		
mouth base	99.4	82.3–116.5		

Parameter	Median survival (OS) [weeks]	95% CI (OS)	Log-rank p (OS)	Log-rank p (RFS)
palate	80.6	0.0–163.9		
tonsil	148.4	0.0–336.7		
oropharynx	21.6	6.3–36.8		
sinus piriformis	44.7	0.0–105.8		
hypopharynx	244.9	111.8–378.0		
larynx	214.6	123.1–306.0		
P16 negative	120.6	82.4–158.8	0.316	0.187
P16 positive	223.6	99.2–348.0		
Ki67 < 50%	125.1	74.5–175.8	0.803	0.396
Ki67 ≥ 50%	107.6	53.4–161.8		
No primary RCT	170.0	93.5–246.5	0.020	0.855
Primary RCT	87.6	41.7–133.5		
No primary radiatio	148.4	89.7–207.2	0.677	0.522
primary radiatio	108.7	54.2–163.3		
No surgery	63.6	25.6–101.5	< 0.001	0.995
surgery	262.9	165.5–360.2		
No adjuvant RCT	121.4	79.0–163.9	0.082	0.942
adjuvant RCT	277.4	111.2–443.7		--
No chemotherapy (all)	139.9	64.0–215.7	0.702	0.744
Chemotherapy (all)	121.6	47.1–196.9		
No adjuvant radiatio	126.3	77.0–175.6	0.528	0.132
adjuvant radiatio	177.1	0.0–531.9		
No adjuvant RCT, cumulative Pt dose	124.3	79.3–169.3	0.047	0.566
adjuvant RCT, cumulative Pt dose	236.1	0.0–481.5		

Parameter	Median survival (OS) [weeks]	95% CI (OS)	Log-rank p (OS)	Log-rank p (RFS)
No palliative radiatio	170.0	107.0–233.0	< 0.001	< 0.001
Palliative radiatio	72.7	56.1–89.3		
L1CAM negative	276.9	164.9–388.8	< 0.001	0.054
L1CAM positive	94.6	69.6–119.5		

Overall Survival by Kaplan-Meier analysis

For this cohort, death was reported for 169 cases (27.5%). L1CAM-, UICC-, N-status and age (> 70) were negative prognostic factors for OS. (Table 2, Fig. 2). In contrast, there was no difference in OS for sex. Alcohol- and tobacco abuse also resulted in unfavorable OS, but for nicotine consumption, this was only a trend (p = 0.106). Overall tumor location was significant for OS (p = 0.003). P16-status, the surrogate marker for HPV infection, was associated with better OS, but this was not significant (p = 0.316). Nevertheless, when we stratified p16-status for tumor location, a higher significance was found for the base of the tongue (n = 29, p = 0.08) and mouth base (n = 15, p = 0.064). Furthermore, patients who underwent surgery and primary RCT had a better OS. Results from the Kaplan-Meier analysis are summarized in Table 2.

Impact of L1CAM expression on survival

L1CAM-status correlated highly significantly with OS, and there was a statistical trend for RFS in the Kaplan-Meier analysis (Fig. 2A, B). We also investigated the influence of L1CAM status in important subgroups. In most of these subgroup analyses, L1CAM was significantly negatively correlated with survival for both alternatives. This was particularly observed for alcohol- and nicotine users as well as p16-status, application of RCT and surgical treatment.

However, for N- as well as UICC-status, L1CAM was only significant for OS in low-risk cases (N0 and UICC I,II respectively) (Fig. 2C-F).

Survival analysis by Cox regression

We complemented the Kaplan-Meier survival analysis by uni- and multivariable Cox regression analysis (Cox Proportional Hazards Survival Regression). The results are summarized in Tables 3 and 4 and Fig. 3. The results obtained were highly similar to the data from the Kaplan-Meier analysis. For RFS, old age (> 70) was significant, and only statistical trends were found for lymph-node metastasis, UICC- and L1CAM-status. Only age and lymph node metastasis remained as independent prognostic factors in multivariable analysis. For OS, in contrast, age, alcohol consumption, tumor size, N-status, high UICC, and L1CAM-status were significant in this analysis. In multivariable Cox regression for OS, age, tumor

size (T), lymph-node metastasis (N) and L1CAM-status turned out to be independent prognostic markers.

Table 3

Cox regression survival analysis for OS and RFS * Included factors were TNM8 (I + II vs. II + IV), age, L1CAM (pos. / neg.), p16, tobacco, alcohol, tumor size, lymph node metastasis (N), grading in forward conditional mode. Factors were included if p < 0.1 and excluded if p > 0.2. Only factors having a p < 0.1 are shown.

Variables	**Univariable analysis OS**				**Multivariable analysis OS***			
	Sig.	**HR**	**95% CI for HR**		**Sig.**	**HR**	**95% KI for HR**	
			lower	upper			lower	upper
Age	0.018	1.021	1.003	1.038	0.001	1.035	1.013	1.065
Gender (female vs. male)	0.561	1.124	0.757	1.669				
Tobacco use (No vs. Yes)	0.108	1.44	0.92	2.27	0.097	1.634	1.914	2.921
Alcohol use (No vs. Yes)	0.017	1.45	1.06	1.98				
P16 (No vs. Yes)	0.317	0.83	0.58	1.18				
Tumor size	< 0.001	1.38	1.19	1.58	0.002	1.288	1.093	1.516
Lymph node metastasis	0.005	1.25	1.07	1.47	0.024	1.56	1.06	2.296
TNM8_1_2 vs. TNM8_3_4	0.001	1.83	1.28	2.62				
Grading	0.255	1.169	0.893	1.528				
L1CAM (No vs. Yes)	< 0.001	1.8	1.3	2.4	0.012	1.595	1.106	2.300
	Univariable analysis RFS				Multivariable analysis RFS*			
	Sig.	HR	95% CI for HR		Sig.	HR	95% CI for HR	
			lower	upper			lower	upper
Age	0.057	1.026	0.999	1.054	0.009	1.043	1.010	1.076
Gender (female vs. male)	0.614	0.854	0.463	1.575				
Tobacco use (No vs. Yes)	0.651	0.860	0.448	1.652				
Alcohol use (No vs. Yes)	0.899	1.033	0.627	1.701				
P16 (No vs. Yes)	0.191	0.643	0.332	1.246				
Tumor size	0.116	1.191	0.958	1.418				
Lymph node metastasis	0.050	1.734	0.999	3.010	0.019	2.136	1.131	4.033
TNM8_I,II vs. TNM8_III,IV	0.099	1.613	0.914	2.846				
Grading	0.278	1.277	0.821	1.987				
L1CAM (No vs. Yes)	0.057	1.635	0.986	2.711				

Discussion

In this monocentric, retrospective study, we examined the prognostic value of L1CAM expression for HNSCC and its association with risk factors, localization and staging. Clinicopathological parameters and prognostic factors in our cohort are similar to previously published data on investigated HNSCC cohorts [18]. We therefore regard our cohort as representative for this entity. As in other cohorts [19, 20], significant prognostic factors for survival (OS) in our cohort were age, tobacco- and alcohol consumption, UICC stage, and N-status. However, for some of these well-known prognostic factors, we only observed a statistical trend in survival analysis. We suggest that this is due to the limited number of cases available. Especially for RFS, the significance level was generally lower due to a lower number of reported relapses, resulting in reduced statistical power. Especially for smoking, we expected a more pronounced prognostic impact. We think that this is related to the lack of detailed data on the intensity of smoking habits in this study. p16 was also not significant in survival analysis when analyzing all cases of the cohort together. However, p16 is known to be more important for the prognosis of the oropharyngeal [21] location. In contrast to other studies, we included all HNSCC locations in our cohort. Indeed, when analyzing the localizations separately for p16-status and OS, we found a statistical trend for the base of tongue (n = 28, log-rank p = 0.080) and mouth base (n = 16, p = 0.064) but not for larynx (n = 64, p = 0.144). This observation is consistent with the view that HNSCC exhibits distinct characteristics [22] across different locations, but this should be investigated further with independent, larger cohorts.

L1CAM staining in tissue showed heterogeneous expression. Especially, we observed a high L1CAM staining intensity at the invasion front. This staining pattern suggests a function of L1CAM in the invasion process as already proposed [23–25]. Due to the heterogeneous staining and as not all specimens contained an invasion front, we decided to score L1CAM-status similarly to the estrogen receptor in breast cancer [26], using a low threshold for intensity (≥ 1) and also percentage of positive cells (> 1%).

With this threshold, L1CAM status was strongly prognostic for unfavorable OS but correlated less significantly with RFS. L1CAM status also correlated well with alcohol uptake, tumor size and UICC stage - all well-known prognostic factors. Noteworthy, in Cox regression analysis, L1CAM-status turned out to be an independent prognostic marker for OS, together with age and high UICC stage (Table 3). However, in cases of advanced UICC stage, the significance of L1-CAM status on OS was markedly lower (Fig. 2). The additional determination of the L1CAM status in low-risk cases could therefore be valuable for diagnostic purposes.

We observed a strong association between alcohol consumption and L1CAM status, while the risk factors tobacco use and p16 (HPV) showed no such relationship. We speculate that high alcohol intake may lead to recurrent tissue damage and thereby cause up-regulation of genes such as L1CAM, but this hypothesis should be investigated further.

L1CAM-status was also different for the tumor localizations, ranging from 67% positive tumors in the hypopharynx to 40% in the larynx or base of the mouth. In contrast to the other locations, we observed a

positive effect of high L1CAM expression in survival analysis in oropharyngeal tumors (n = 23, p = 0.023) for OS (Table 1), highlighting the different behavior of HNSCCs in the localizations, as found for p16-status.

Although L1CAM expression was proposed to be involved in the invasion process [27] and we observed strong staining at the invasion front, we could not find a significant association with lymph node metastasis (N). Thus, L1CAM might promote tumor progression by a mechanism other than invasion into the lymph vessels. Gavert et al. [28] investigated the role of L1CAM and beta-catenin expression in colorectal carcinomas. On the molecular level, beta-catenin serves as an adhesion linker of cadherin receptors to the cytoskeleton and as a key transducer of Wnt signaling pathway to the nucleus [29]. Gavert et al found that co-expression of nuclear beta-catenin and L1CAM occurred mainly at the invasion front and not in central, more differentiated parts of the tumor. Since L1CAM contains an ADAM10/17- and a matrix metalloprotease 16 (MMP16) cleavage site, L1CAM shedding will probably occur mostly at the invasion front, promoting the dissemination of colon cancer cells. Another finding of Gavert et al. was the close vicinity of an L1CAM positive invasion front to nerve axons, which supports the assumption that tumor cells use axonal guidance for spreading. This perineural invasion (PNI) was also described by Trietsch et al. [30] concerning vulvar squamous cell carcinomas and by Karpinkska et al. [31] for HNSCC. We found only rare events of PNI in our cohort. Nevertheless, further examination of PNI and beta-catenin could improve our understanding of L1CAM's impact on metastasis of HNSCC. As downstream signaling of L1CAM involves several pathways, such as MAPK, PI3K, ERK, Rac-1 and NF-kB [29], we propose to investigate the activity of these pathways at the invasion front in more detail.

Altogether, our survival and correlation data further support the observation that high expression of L1CAM is associated with significantly higher UICC stages [27] in HNSCC and also an unfavorable prognosis, but is also an independent prognostic marker as shown by multivariable Cox regression analysis.

Treatment options for HNSCC are primary surgery, primary RCT, primary radiotherapy, and adjuvant RCT and adjuvant radiotherapy. Immunotherapy was introduced in 2016 for recurrent cases and in 2019 as first-line therapy [32]. All treatments are chosen according to the characteristics of the individual tumor and patient [33]. In our cohort, immunotherapy was not applied as we stopped including patients in 2020 to obtain a reasonable follow-up period. Any association of therapy treatments such as surgery or palliative radiotherapy with L1CAM status will be confounded by the factors determining the therapy decision.

In our cohort, primary surgery was the most frequently applied and effective option regarding OS (Table 2). As patients with HNSCC are often diagnosed with lymph node metastasis at first examination, it is expected that radiotherapy, chemotherapy or both (RCT) would further decrease cancer cell load. However, primary RCT did not reach a similar statistical significance on OS as surgery in our cohort. Adjuvant RCT did not reach the significance level, yet there was a trend with p = 0.063, which could be

due to the low number of cases (n = 38). Palliative treatment is chosen for patients in late stages of HNSCC, and this treatment is therefore associated with bad outcome.

Xie et al. described a synergistic effect of Integrin-β1 and L1CAM increasing resistance to chemotherapy for esophageal squamous cell cancer via the AKT pathway [34]. Knockdown of both molecules decreased chemoresistance to cisplatin. We could not detect an effect of L1CAM on survival in relation to chemotherapy in our cohort. Nevertheless, these findings could be a valid starting point for future research on HNSCC.

Limitations:

Since we investigated a retrospective monocentric study, the data collection depended on sufficient and thorough clinical examinations. Calculations were limited by missing data in the hospital's clinical data system, e.g., follow-up, cause of death, treatments, RCT regime, and relapse. Furthermore, the study relies solely on immunohistochemistry and does not take other independent techniques and molecular parameters into account. Our cohort is also heterogeneous and comprises several anatomical tumor locations. The four anatomical site groups and the nine separately considered locations represent different physiological areas and are therefore heterogeneous in function and protein expression pattern. The statistical power of this study is therefore limited when subgroups are evaluated separately.

Conclusions

Our study confirms that L1CAM is closely associated with lower overall survival in HNSCC. Alcohol consumption appeared to be the main risk factor for L1CAM expression. Since established biomarkers such as p16 status and Ki67 are not associated with L1CAM, a correlation with PD-L1 could be part of future examination. Higher T-stages and UICC stages correlated with L1CAM positivity, whereas this effect could not be found for lymph node metastasis and distant metastasis. For diagnostic purposes, L1CAM could be a suitable prognostic marker in earlier UICC stages. In subgroup analysis, a more detailed examination of the impact of L1CAM on larynx carcinomas should be implemented. In our cohort, relapses were not significantly associated with L1CAM. Primary surgery was shown to be the most effective treatment option for HNSCC. Adjuvant treatment options did not reach statistical significance; however, further molecular research regarding integrin beta 1 could provide more detailed information. Another prospective study aim could be the examination of L1CAM and PNI as well as immunoglobulin-like domains as specific targets for antibody therapy.

Declarations

Ethics approval and consent to participate

The ethics commission of our institution, Brandenburg Medical School (MHB-Theodor Fontane, Campus Rüdersdorf, Seebad 82/83, 15562 Rüdersdorf bei Berlin, Germany; file number: E-01-20211028),

approved this study. All experiments and procedures were performed according to the current guidelines, especially the Declaration of Helsinki. Informed consent was waived due to the retrospective character of this study and the disproportionately high effort for contacting the patients after the follow-up period.

Patient consent for publication

Not applicable

Competing interests

The authors state that no competing interests exist.

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Author Contribution

NN did data curation, statistical analysis, manuscript writing; TK contributed data collection, supervision, manuscript editing, study design; LM did data collection, data analysis manuscript editing, MR: contributed study design, supervision, manuscript editing; S-S-S did supervision, study design, manuscript editing. All authors reviewed the manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

References

1. Head and neck squamous cell carcinomas. Onkopedia n.d. https://www.onkopedia.com/en/onkopedia/guidelines/head-and-neck-squamous-cell-carcinomas

(accessed September 17, 2025).

2. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6:92. https://doi.org/10.1038/s41572-020-00224-3.
3. Glastonbury CM. Head and Neck Squamous Cell Cancer: Approach to Staging and Surveillance. In: Hodler J, Kubik-Huch RA, von Schulthess GK, editors. Diseases of the Brain, Head and Neck, Spine 2020–2023: Diagnostic Imaging. Cham (CH): Springer; 2020.
4. Budu VA, Decuseară T, Balica NC, Mogoantă CA, Rădulescu LM, Chirilă M, et al. The role of HPV infection in oropharyngeal cancer. Rom J Morphol Embryol. 2019;60:769–73.
5. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol. 2015;8:11884–94.
6. Chow LQM. Head and Neck Cancer. N Engl J Med. 2020;382:60–72. https://doi.org/10.1056/NEJMra1715715.
7. Pai SI, Westra WH. Molecular Pathology of Head and Neck Cancer: Implications for Diagnosis, Prognosis, and Treatment. Annu Rev Pathol. 2009;4:49–70. https://doi.org/10.1146/annurev.pathol.4.110807.092158.
8. Eberly HW, Sciscent BY, Lorenz FJ, Rettig EM, Goyal N. Current and Emerging Diagnostic, Prognostic, and Predictive Biomarkers in Head and Neck Cancer. Biomedicines. 2024;12:415. https://doi.org/10.3390/biomedicines12020415.
9. Redman-Rivera LN, Shaver TM, Jin H, Marshall CB, Schafer JM, Sheng Q, et al. Acquisition of aneuploidy drives mutant p53-associated gain-of-function phenotypes. Nat Commun. 2021;12:5184. https://doi.org/10.1038/s41467-021-25359-z.
10. Lin Y-M, Sung W-W, Hsieh M-J, Tsai S-C, Lai H-W, Yang S-M, et al. High PD-L1 Expression Correlates with Metastasis and Poor Prognosis in Oral Squamous Cell Carcinoma. PLoS ONE. 2015;10:e0142656. https://doi.org/10.1371/journal.pone.0142656.
11. Flaviana C, Monica P, Terenzio C, Raffaele M, Valentina A, Giulia C, et al. L1CAM expression in human gastrointestinal tract development: From tongue to colon-rectum. J Public Health Res. 2023;12:22799036231165624. https://doi.org/10.1177/22799036231165624.
12. Kiefel H, Bondong S, Hazin J, Ridinger J, Schirmer U, Riedle S, et al. L1CAM. Cell Adh Migr. 2012;6:374–84. https://doi.org/10.4161/cam.20832.
13. van der Maten M. L1 Cell Adhesion Molecule in Cancer, a Systematic Review on Domain-Specific Functions n.d.
14. AWMF. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF): S3-Leitlinie Diagnostik, Therapie, Prävention und Nachsorge des Oro- und Hypopharynxkarzinoms, Langversion 1.0 2024. https://register.awmf.org/de/leitlinien/detail/032-034OL (accessed June 16, 2026).
15. Guo M, Gong H, Nie D, Li Z. High L1CAM expression predicts poor prognosis of patients with endometrial cancer. Med (Baltim). 2021;100:e25330. https://doi.org/10.1097/MD.0000000000025330.

16. de Freitas D. L1 Cell Adhesion Molecule (L1CAM) expression in endometrioid endometrial carcinomas: A possible pre-operative surrogate of lymph vascular space invasion n.d.
17. Zanoni DK, Patel SG, Shah JP. Changes in the 8th Edition of the American Joint Committee on Cancer (AJCC) Staging of Head and Neck Cancer: Rationale and Implications. Curr Oncol Rep. 2019;21:52. https://doi.org/10.1007/s11912-019-0799-x.
18. Economopoulou P, de Bree R, Kotsantis I, Psyrri A. Diagnostic Tumor Markers in Head and Neck Squamous Cell Carcinoma (HNSCC) in the Clinical Setting. Front Oncol. 2019;9:827. https://doi.org/10.3389/fonc.2019.00827.
19. Alotaibi M, Valova V, HÄnsel T, Stromberger C, Kofla G, Olze H, et al. Impact of Smoking on the Survival of Patients With High-risk HPV-positive HNSCC: A Meta-analysis. Vivo. 2021;35:1017–26. https://doi.org/10.21873/invivo.12345.
20. Kim DH, Kim SW, Han JS, Kim G-J, Basurrah MA, Hwang SH. The Prognostic Utilities of Various Risk Factors for Laryngeal Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis. Med (Kaunas). 2023;59:497. https://doi.org/10.3390/medicina59030497.
21. Priyatha V, Gupta H, Narsapuram P, Ahmed F, Alhussain H, Arfa A, et al. Significance of p16 in Site-Specific Human Papillomavirus-Positive and Negative Head and Neck Squamous Cell Carcinoma (HNSCC). Cureus. 2024;16:e63594. https://doi.org/10.7759/cureus.63594.
22. Du E, Mazul AL, Farquhar D, Brennan P, Anantharaman D, Abedi-Ardekani B, et al. Long-term Survival in Head and Neck Cancer: Impact of Site, Stage, Smoking, and Human Papillomavirus Status. Laryngoscope. 2019;129:2506–13. https://doi.org/10.1002/lary.27807.
23. Kiefel H, Bondong S, Hazin J, Ridinger J, Schirmer U, Riedle S, et al. L1CAM: a major driver for tumor cell invasion and motility. Cell Adh Migr. 2012;6:374–84. https://doi.org/10.4161/cam.20832.
24. van der Maten M, Reijnen C, Pijnenborg JMA, Zegers MM. L1 Cell Adhesion Molecule in Cancer, a Systematic Review on Domain-Specific Functions. Int J Mol Sci. 2019;20:4180. https://doi.org/10.3390/ijms20174180.
25. Chien M-H, Yang Y-C, Ho K-H, Ding Y-F, Chen L-H, Chiu W-K, et al. Cyclic increase in the ADAMTS1-L1CAM-EGFR axis promotes the EMT and cervical lymph node metastasis of oral squamous cell carcinoma. Cell Death Dis. 2024;15:82. https://doi.org/10.1038/s41419-024-06452-9.
26. Salama AM, Hanna MG, Giri D, Kezlarian B, Jean M-H, Lin O, et al. Digital Validation of Breast Biomarkers (ER, PR, AR and HER2) in Cytology Specimens Using Three Different Scanners. Mod Pathol. 2022;35:52–9. https://doi.org/10.1038/s41379-021-00908-5.
27. Kim J-H, Lee K-W, Ahn D-G, Oh K-Y, Yoon H-J. Clinical significance of L1CAM expression and its biological role in the progression of oral squamous cell carcinoma. Oncol Rep. 2023;49:67. https://doi.org/10.3892/or.2023.8504.
28. Gavert N, Conacci-Sorrell M, Gast D, Schneider A, Altevogt P, Brabletz T, et al. L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol. 2005;168:633–42. https://doi.org/10.1083/jcb.200408051.

29. Cheriyamundath S. Ben-Ze'ev A. Wnt/β-Catenin Target Genes in Colon Cancer Metastasis: The Special Case of L1CAM. Cancers (Basel). 2020;12:3444. https://doi.org/10.3390/cancers12113444.
30. Trietsch MD, Oonk MHM, Hawinkels LJAC, Bor R, van Eendenburg JDH, Ivanova Z, et al. Prognostic value and clinicopathologic characteristics of L1 cell adhesion molecule (L1CAM) in a large series of vulvar squamous cell carcinomas. Oncotarget. 2016;7:26192–205. https://doi.org/10.18632/oncotarget.8353.
31. Karpińska K, Gielata M, Gwiazdowska A, Boryń Ł, Kobielak A. Catulin Based Reporter System to Track and Characterize the Population of Invasive Cancer Cells in the Head and Neck Squamous Cell Carcinoma. Int J Mol Sci. 2021;23:140. https://doi.org/10.3390/ijms23010140.
32. Liu X, Harbison RA, Varvares MA, Puram SV, Peng G. Immunotherapeutic strategies in head and neck cancer: challenges and opportunities. J Clin Invest. 2025;135:e188128. https://doi.org/10.1172/JCI188128.
33. Bhatia A, Burtness B. Treating Head and Neck Cancer in the Age of Immunotherapy: A 2023 Update. Drugs. 2023;83:217–48. https://doi.org/10.1007/s40265-023-01835-2.
34. Xie Y-H, Ran L-Q, Wu Z-Y, Sun C, Xu X-E, Zou H-Y, et al. Role of Integrin β1 in the progression and chemo-resistance of esophageal squamous cell carcinoma. J Cancer. 2022;13:2074–85. https://doi.org/10.7150/jca.68647.

Figures

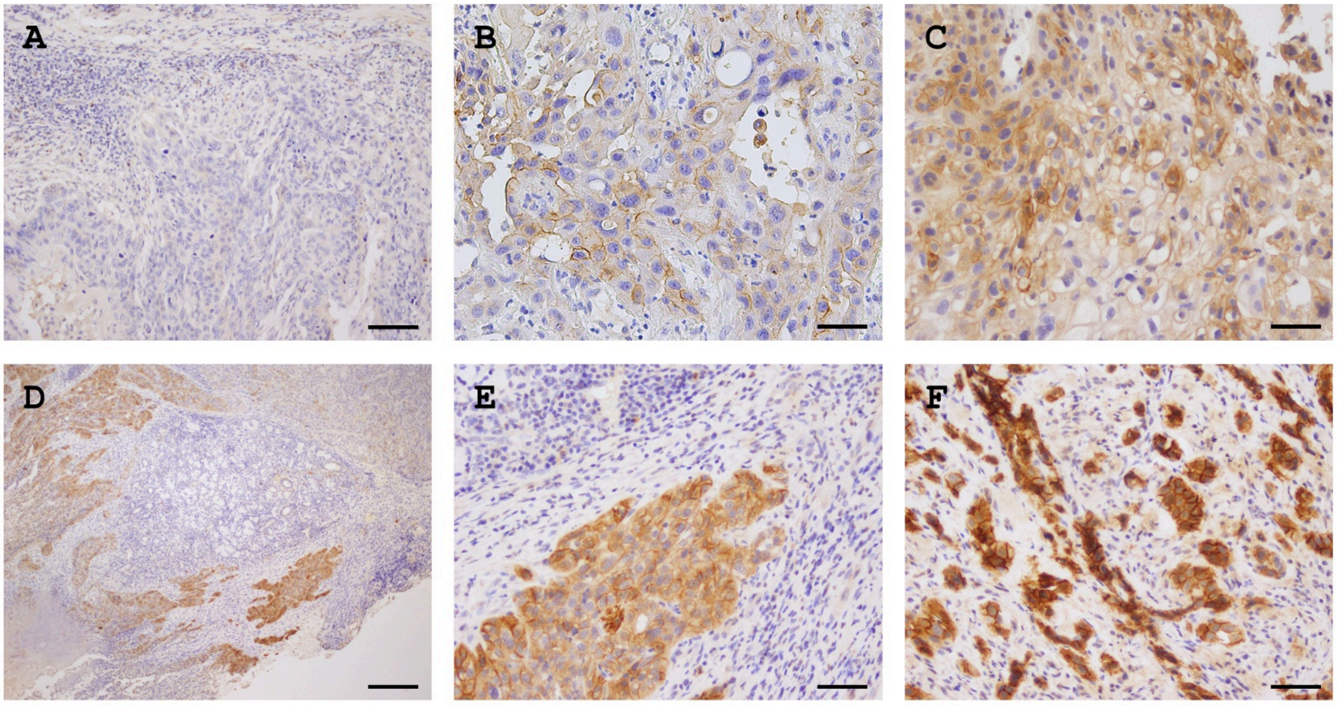


Figure 1

L1CAM immunohistochemistry in cases of squamous cell carcinomas

A: negative staining, bar = 100 µm; B: weak staining, bar = 50 µm; C: moderate staining, bar = 50 µm; D: stronger staining at the invasion front, bar = 200 µm; E: same case, higher magnification showing moderate staining, bar = 50 µm; F: strong staining, bar = 50 µm.

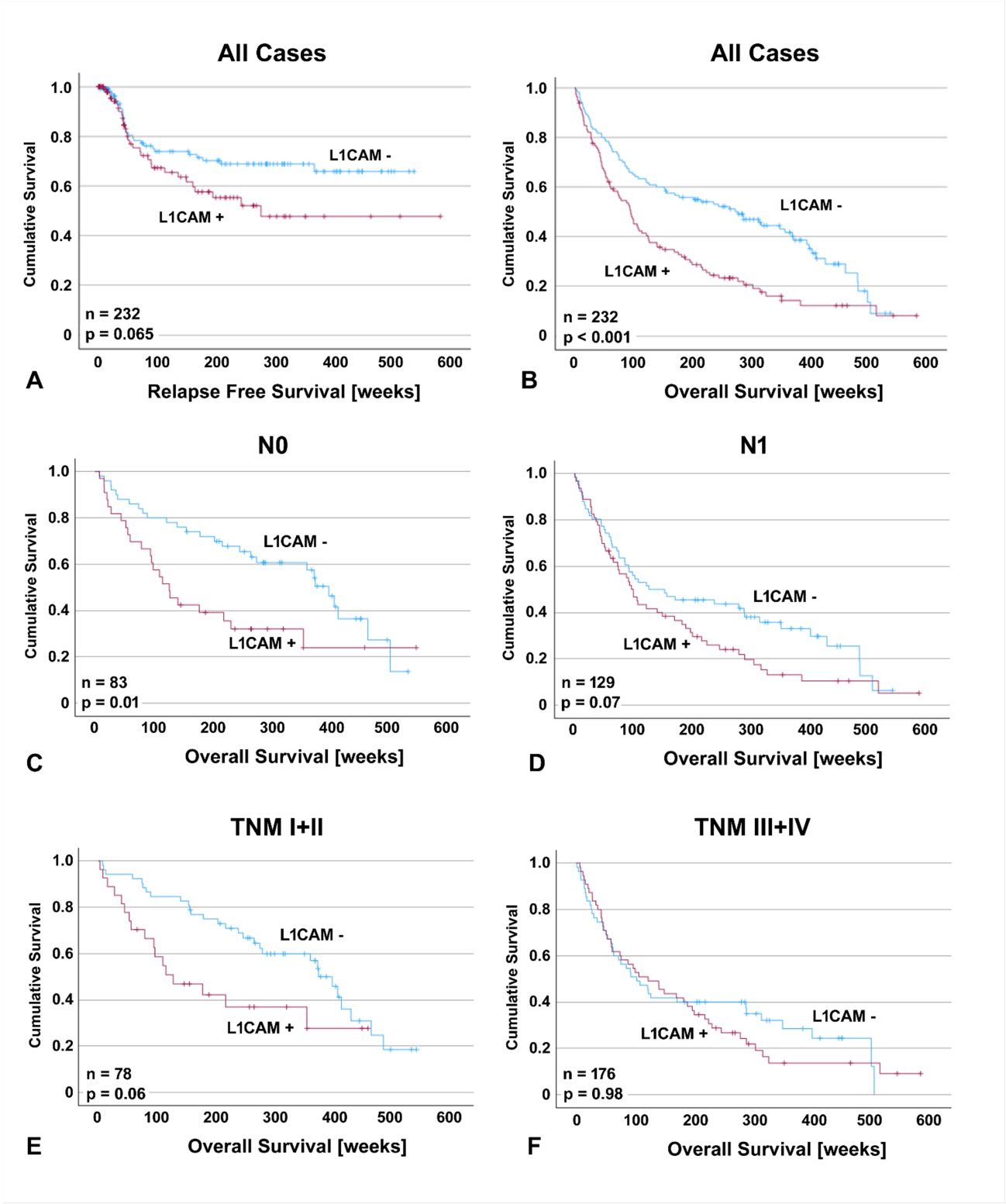


Figure 2

Survival analysis by Kaplan-Meier analysis for overall survival and relapse-free survival for all cases. A. Relapse-free survival, all cases included, B-F: Overall survival, B: all cases, C: Cases without lymph node infiltration, D cases with lymph node metastasis, E: cases with low staging (I,II), F: cases with higher staging (III,IV). Number of cases is included (n) and log-rank p (p) given.

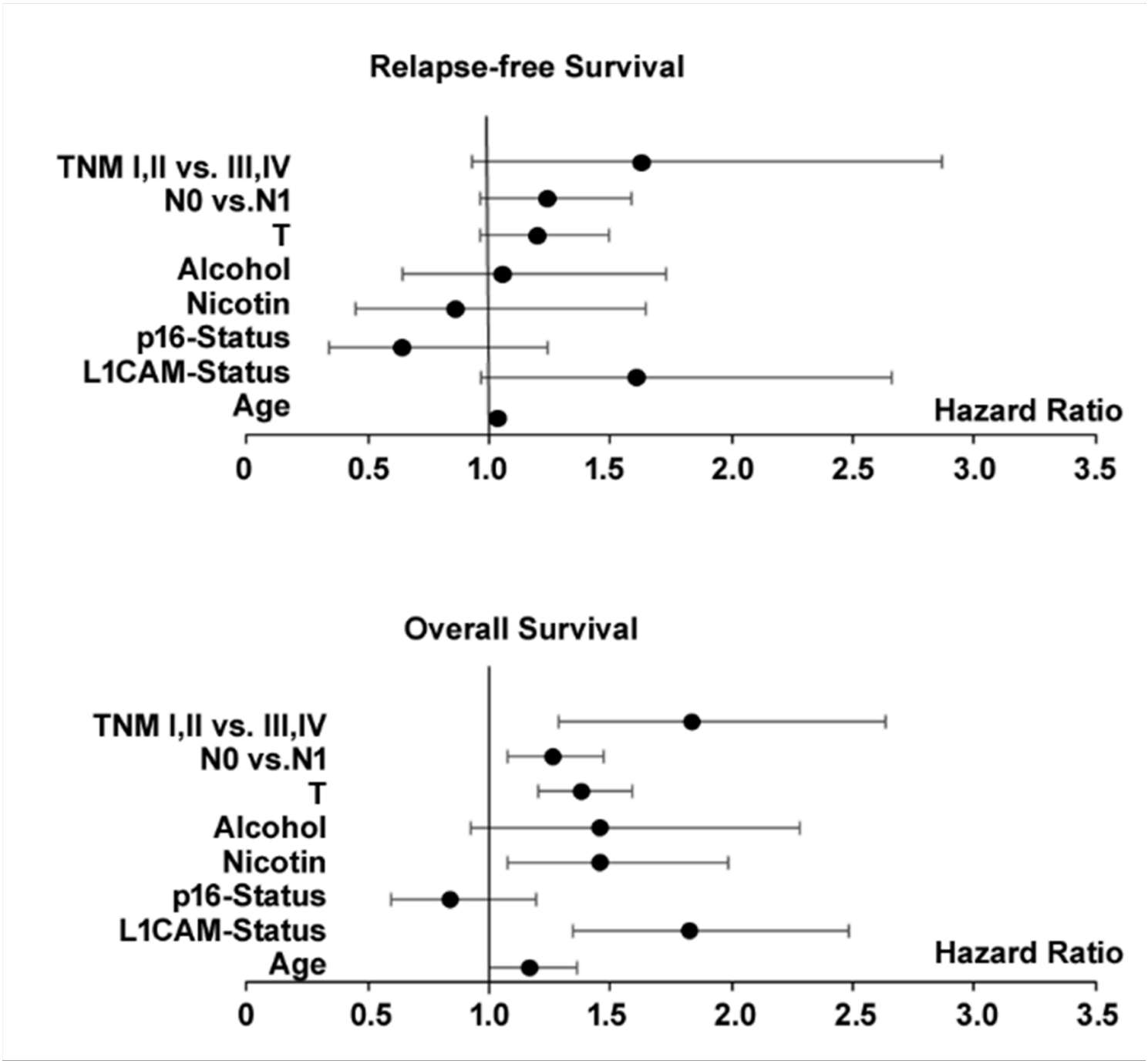


Figure 3

Forest plot summarizing the results of univariable cox regression survival analysis.

Supplementary Files

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- Table1.docx
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- Table3.docx