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Immune Cell and Biochemical Biomarkers in Advanced Laryngeal Cancer

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Abstract

Objective: The aim of this study was to evaluate cell and biochemical biomarkers and establish their prognostic value in patients with advanced laryngeal cancer.

Material and Methods: A prospective study included 52 patients with advanced laryngeal carcinoma surgically treated at the tertiary referral center. Tumor tissue was immunohistochemically stained for T-cell markers (CD4 and CD8), and levels of cytokines (IL-6 and IL-8) and C-reactive protein were analyzed from blood samples.

Results: Overall 3-year survival (OS) of patients included in the study was 69.2% and the disease specific survival (DSS) 72.5%. Higher expression of $CD4^+$ and $CD8^+$ were significant prognostic factors with positive impact on both OS and DSS in univariate analysis, but not in multivariate analysis. Levels of IL-8 were a significant predictor of 3-year OS and DSS survival in patients with advanced laryngeal cancer but not levels of IL-6 and CRP values.

Conclusion: Though high expression of CD4 and CD8 were demonstrated in the tumor tissue, but their prognostic role was not established. Higher values of IL-8 proved to be significant negative predictor of DSS. This could further collaborate the inclusion of combination of biomarkers in assessment of favorable treatment choice in patients with advanced laryngeal carcinoma.

Keywords

advanced laryngeal cancer, prognostic biomarkers, tumor-infiltrating lymphocytes, cytokines, C-reactive protein

Introduction

Laryngeal carcinomas make 1%–3.5% of all head and neck malignancies.[1] During the past 3 decades, incidence and prevalence of the disease have increased by 12% and 24%, respectively, but mortality has declined by 5%.[2] Most patients with advanced laryngeal cancer require treatment that involves combinations of surgery, radiotherapy, and chemotherapy, with a 5-year survival rate up to 80%.[3]

Researchers have been struggling for years to identify biomarkers that will provide better treatment selection and prognostication for patients with laryngeal carcinoma. Inflammatory response of the immune system and the factors it

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produces are believed to play a key role in laryngeal tumorigenesis, progression, and response to therapy.[4] In the recent years, tumor-infiltrating lymphocytes (TILs) emerged as a possible immune cell biomarker in head and neck squamous cell carcinoma.[5] The quantity of certain TILs, including $CD4^+$ and $CD8^+$, have been associated with improved survival and response to therapy in head and neck cancer.[6,7] Cytokines and circulating inflammatory proteins indicate a presence or an absence of a functional immune response in the tumor microenvironment.[8] Elevated values of interleukins 6 and 8 were detected with higher rate of recurrence and poor survival in patients with head and neck carcinoma.[9-11] C-reactive protein (CRP) is an acute inflammation phase and a part of the innate immune system which generates pro-inflammatory cytokines and enhances the inflammatory response. A chronic low-grade inflammation could also be reflected by moderately elevated levels of CRP and is associated with an increased risk for various cancers.[11–13]

The aim of this study was to evaluate TILs density in the tumor tissue and values of plasma pro-inflammatory cytokines and CRP as cell and biochemical biomarkers in patients with advanced laryngeal cancer. Further, the study wanted to investigate the prognostic significance of these biomarkers could have prognostic significance clinical characteristics and survival rates of these patients.

Material and Method

Patients Selection

A prospective study included 52 patients with squamocellular carcinoma of the larynx surgically treated in the period from April 2019 to April 2022 treated at the tertiary referral center. This study was approved by the Institutional Ethics Committee (152/01-19), and all patients signed the informed consent form prior to their inclusion in the study. The diagnosis of laryngeal carcinoma was confirmed by otorhinolaryngological clinical examination and laryngomicroscopic examination of the larynx with the biopsy and histopathologic examination of the tissue. Additional diagnostics (chest radiography and computed tomography of the neck and ultrasonography of the abdomen) were performed to determine the TNM stage of the disease. Study included patients diagnosed with advanced stages of operable laryngeal carcinoma (T3N0-N2 and T4aN0-N2), without previous treated malignancies and distant metastases. The modality of treatment for every patient was decided on the institutional Oncological Board (consisting of a radiotherapist, head and neck surgeons, an oncologist, and a histopathologist). Choice of primary and adjuvant treatment was decided based on the National Comprehensive Cancer Network (NCCN) and the American Society for Radiation Oncology (ASTRO) guidelines which are recommended and used treatment guidelines at our institution.[14,15] Every patients was surgically treated, which involved resection of the tumor with some form of the neck dissection in case of cervical lymphadenopathy. If it was needed, patients received external radiotherapy with total dose of 60 to 70 Gy in 30–35 fractions for 6–7 weeks, or concomitant chemotherapy consisted of at least three courses of cisplatin (CDDP) with 5-fluorouracil (5-FU) intravenously. Follow-up period was up to 36 months. Demographic, clinical and histopathological characteristics (age and gender, tobacco use, alcohol consumption, histopathological tumor grade, TNM classification, and therapy modality) were noted.

Control group was formed in order to establish normative data for circulatory cytokines (IL-6 and IL-8) and serum CRP values. The group included 45 healthy volunteers (38 men and 7 women; median age 59 years, range 45–76 years) with normal otorhinolaryngological and laryngoscopy findings, without previous diagnoses, and treatment of malignancies.

Tumor-Infiltrating Lymphocytes (TILs)

Tumor tissue was collected from operative laryngeal tissue samples. Sections of the formalin-fixed and paraffin-embedded (FFPE) blocks were stained with hematoxylin and eosin (H&E). Following central evaluation of hematoxylin and eosin (H&E) sections, for each patient three .6 mm tissue cores were obtained from the assigned area of the FFPE blocks and collected in a tissue microarray (TMA). TMA tissue sections (4 μm) were immunohistochemically stained with anti-CD4 rabbit monoclonal antibody (clone SP35, 1:50, Abcam, Cambridge, UK) and anti-CD8 mouse monoclonal antibody (clone C8/144B, 1:100, DAKO, Glostrup, DK) according to manufacturer's instructions. Staining was performed by an IHC slide staining system (BenchMark Special Stains, Ventana Medical Systems, Inc.) TMAs were captured with an Olympus DP70 ×20 objective lens. Cores consisting of <50% tumor parenchyma, partial cores, and those with significant tumor necrosis were excluded from the analysis. Negative control slides in the absence of primary antibodies were included for each staining. The CD4 and CD8 positively immunostained cells were manually counted (at ×200 magnification) using the ImageJ software. Average total number of TILs was presented as total number of detected positive cells per mm^2 of tumor core for triplicate samples for each patient. For statistical purposes the median value was used as the cut-off to define high and low density for both CD4 and CD8.

Measurement of Cytokines

To determine specific circulative cytokines (IL-6, IL-8), blood was taken in anticoagulant-free tubes (9 mL tubes with a red-black cap). After coagulation (20 min), the tubes were centrifuged at 3000 r/min for 10 min at 4°C. After centrifugation, the obtained serum was be pipetted into tubes and stored in a refrigerator at −80°C until further analysis. Serum IL-6 and IL-8 levels were determined by commercial coated ELISA kits, according to manufacturer instructions (Elabscience ELISA kit; Huston, USA). For statistical purposes, the

Table 1. Patient characteristics with distribution of TILs, circulatory cytokines and CRP levels.

		CD4 n (%)			CD8 n (%)			IL-6 n (%)			IL-8 n (%)			CRP n (%)		
Patient Characteristics		Low	High	*p*	Low	High	*p*	Low	High	*p*	Low	High	*p*			*p*
Age	1 (1.9)	1 (1.9)	0 (0)	.744	1 (1.9)	0 (0)	.338	0 (0)	1 (1.9)	.451	0 (0)	1 (1.9)	.246	1 (1.9)	0 (0)	.099
<45	8 (15.4)	4 (7.7)	4 (7.7)		3 (5.8)	5 (9.6)		6 (11.5)	2 (3.8)		5 (9.6)	3 (5.8)		6 (11.5)	2 (3.8)	
45-54	24 (46.2)	14 (26.9)	10 (19.2)		14 (26.9)	10 (19.2)		11 (21.1)	13 (25)		12 (23.1)	12 (23.1)		17 (32.7)	7 (13.5)	
55–64	16 (30.8)	7 (13.5)	9 (17.3)		8 (15.4)	8 (15.4)		9 (17.3)	7 (13.5)		10 (19.2)	6 (11.5)		8 (15.4)	8 (15.4)	
65-74	3 (5.8)	2 (3.8)	1 (1.9)		3 (5.8)	0 (0)		1 (1.9)	2 (3.8)		0 (0)	3 (5.8)		0 (0)	3 (5.8)	
>75																
Gender n (%) *male female*	47 (90.4)	25 (48.1)	22 (42.3)	.772	25 (48.1)	22 (42.3)	.251	24 (46.2)	23 (44.2)	.704	25 (48.1)	22 (42.3)	.575	30 (57.7)	17 (32.7)	.298
	5 (9.6)	3 (5.8)	2 (3.8)		4 (7.7)	1 (1.9)		3 (5.8)	2 (3.8)		2 (3.8)	3 (5.8)		2 (3.8)	3 (5.8)	
Smoking n (%) *smokers*	44 (84.6)	22 (42.3)	22 (42.3)	.192	24 (46.1)	20 (38.5)	.677	24 (46.2)	20 (19.2)	.375	25 (48.1)	19 (36.5)	.098	29 (55.8)	15 (28.8)	.129
non-smokers	8 (15.4)	6 (11.5)	2 (3.8)		5 (9.6)	3 (5.8)		3 (5.8)	5 (9.6)		2 (3.8)	6 (11.5)		3 (5.8)	5 (9.6)	
Histological grade n (%)	11 (21.2)	6 (11.5)	5 (9.6)	.287	7 (13.5)	4 (7.7)	.66	5 (9.6)	6 (11.5)	.497	7 (13.5)	4 (7.7)	.434	5 (9.6)	6 (11.5)	.296
G1	35 (67.3)	17 (32.7)	18 (34.6)		18 (34.6)	17 (32.7)		20 (19.2)	15 (28.8)		16 (30.8)	19 (36.5)		22 (42.3)	13 (25)	
G2	6 (11.5)	5 (9.6)	1 (1.9)		4 (7.7)	2 (3.8)		2 (3.8)	4 (7.7)		4 (7.7)	2 (3.8)		5 (9.6)	1 (1.9)	
G3																
Tumor localization n (%)	13 (25)	7 (13.5)	6 (11.5)	.596	9 (17.3)	4 (7.7)	.526	6 (11.5)	7 (13.5)	.532	6 (11.5)	7 (13.5)	.831	7 (13.5)	6 (11.5)	.521
Supraglottis	12 (23.1)	5 (9.6)	7 (13.5)		6 (11.5)	6 (11.5)		5 (9.6)	7 (13.5)		7 (13.5)	5 (9.6)		9 (17.3)	3 (5.8)	
Glottis	27 (51.9)	16 (30.8)	21 (40.4)		14 (26.9)	13 (25)		16 (30.8)	11 (21,2)		14 (26.9)	13 (25)		16 (30.8)	11 (21.1)	
Transglottic																
T Stage n (%)	50 (96.2)	26 (50)	24 (46.2)	.182	27 (51.9)	23 (44.2)	.199	27 (51.9)	23 (44.2)	.134	27 (51.9)	23 (44.2)	.134	31 (59.6)	19 (36.5)	.732
T3	2 (3.8)	2 (3.8)	0 (0)		2 (3.8)	0 (0)		0 (0)	2 (3.8)		0 (0)	2 (3.8)		1 (1.9)	1 (1.9)	
T4a																
N stage n (%)	39 (75)	19 (36.5)	20 (38.5)	.421	18 (34.6)	21 (40.4)	.048	20 (38.5)	19 (36.5)	.377	22 (42.3)	17 (32.7)	.096	25 (48.1)	14 (26.9)	.292
N*0*	9 (17.3)	6 (11.5)	3 (5.8)		8 (15.4)	1 (1.9)		6 (11.5)	3 (5.8)		5 (9.6)	4 (7.7)		6 (11.5)	3 (5.8)	
N*1*	4 (7.7)	3 (5.8)	1 (1.9)		3 (5.8)	1 (1.9)		1 (1.9)	3 (5.8)		0 (0)	4 (7.7)		1 (1.9)	3 (5.8)	
N2																
Treatment n (%) *OP+RT*	1 (1.9)	0 (0)	1 (1.9)	.54	0 (0)	1 (1.9)	.504	1 (1.9)	0 (0)	.347	1 (1.9)	0 (0)	.347	1 (1.9)	0 (0)	.654
OP+RT+CH	47(90.4)	26 (50)	21 (40.4)		27 (51.9)	20 (38.5)		25 (48.1)	22 (42.3)		25 (48.1)	22 (42.3)		29 (55.8)	18 (34.6)	
	4 (7.7)	2 (3.8)	2 (3.8)		2 (3.8)	2 (3.8)		1 (1.9)	3 (5.8)		1 (1.9)	3 (5.8)		2 (3.8)	2 (3.8)	

IL-6- Interleukin 6, IL-8- Interleukin 8, CRP-C reactive protein, HR – hazard ratio; CI – confidence interval; OP – surgery; RT – radiotherapy; CH – chemoradiotherapy.

median value was used as the cut-off to define high and low concentration for both IL-6 and IL-8.

Measurement of Plasma CRP

For establishing plasma CRP levels, .6 mL blood was drawn to a green top (Li Heparin) tube and analyzed using a Nycocard CRP kit (Abbott laboratories, Chicago, USA) according to the manufacturer's protocol on a Cobas 501 analyzer (Rosche Diagnostics, Basel, Switzerland). Value of 3.0 mg/L was used as a the cut-off value

Statistical Analysis

For statistical analysis of data, the program SPSS v20 (Statistical Package for Social Sciences, SPSS Inc, Chicago, Illinois) was used. Descriptive statistics were used for demographic characteristics, risk factors, and other parameters and presented as frequencies and proportions. Pearson and Spearman's Rho Correlation was used to establish the correlation between some demographic and clinical parameters and examined biomarkers. Overall survival (OS) and disease specific survival (DSS) rates were calculated using the Kaplan–Meier method. A Cox proportional hazards regression model along with univariate and multivariate analyses were used for estimating the impact of prognostic factors on OS and DSS rate. Risk estimates are presented as hazard ratios (HR) with 95% confidence intervals (CI). Statistical significance was considered at $p < .05$.

Results

The study included 47 (90.4%) males and 5 (9.6%) females of an median age of 61 years (range 41–77). Among patients, 44 (84.6%) regularly consumed cigarettes. Majority of carcinomas were histologically moderately differentiated tumors (67.3%) and transglottic tumors (51.9%) Most of the patients were treated with surgery followed by postoperative radiotherapy (92.3%), while there were only small number of patients treated only surgically (1.9%) or with surgery with concomitant chemo-radiotherapy (7.7%) (Table 1)

The mean number of positively stained CD4 per mm^2 was 594.8 (median=679.5, range 40–1022), and CD8 per mm^2 was 849.4 (median=799.5, range 49–2001). Using median value as a cut-off, CD4 expression was defined as "low" (when bellow and equal to median value) in 28 patients (53.8%) and "high" (when above median value) in 24 patients (46.2%). CD8 expression was defined as "low" in 29 patients (55.8%) and "high" in 23 patients (44.2%). CD8 expression in N0 stage was significantly higher comparing to other N stages of the disease ($P = .048$). Distribution of CD4 and CD8 expression for different patient characteristics is presented in the Table 1.

Mean value of circulating IL-6 levels was 55.8 pg/mL (median =49, range 16.1–116), and IL-8 levels was 45.1 pg/mL (median=39.4, range 11.7–94.3). These values were significantly higher comparing to those in healthy control group (.96 pg/mL, range 0–4.7) for IL-6 and for IL-8, respectively (.83 pg/mL, range .4–5.1) ($P < .05$) Using median value as a cut-off, circulating levels of IL-6 and IL-8 were defined as "low" in 27 (51.9%) and "high" in 25 (48.1%) of the patients. (Table 1)

Average value of plasma CRP was 4.3 mg/L (median=3, range 0–22), which was significantly higher comparing to control group (.2 mg/L, range 0-2) ($P < .05$). Using median value as a cut-off, values of plasma CRP were defined as "low" in 32 (61.9%) and "high" in 20 (38.5%) of the patients. (Table 1)

A significant positive correlation was found between age and CRP levels (Pearson correlation, $P \leq .05$). A significant negative correlation was found between N stage of the disease and high expression of CD8 in patients with advanced laryngeal cancer (Spearman's Rho, $P \leq .05$). (Table 2)

Survival Analysis and Prognostic Factors

Overall 3-year survival (OS) of patients with advanced laryngeal cancer included in the study was 69.2% and the disease specific survival (DSS) was 72.5%. OS (Log rank $P = .038$) and DSS (Log rank $P = .026$) were significantly higher in patients with high expression of CD4. Also, high expression of CD8 in patients with advanced laryngeal cancer ensured significantly better OS (Log rank $P = .021$) and DSS (Log rank $P = .013$). 3-Year OS (Log rank $P = .318$) and DSS (Log rank $P = .31$) in patients with high values of IL-6 did not differ significantly comparing to these with low values of Il-6 (3-year OS and DSS were 64 and 66.7, respectively, compared to 74.1 and 77.8, respectively). On the other hand, patients with higher values of IL-8 had significantly lower OS (Log rank $P = .012$) and DSS (Log rank $P = .002$) survival comparing to patients with low values of IL-8. Different values of CRP did not significantly influence 3-year survival in patients with advanced laryngeal cancer (log rank, $P > .05$) (Figure 1).

Univariate Cox regression analysis indicated that higher expression of CD4 and CD8 were significant prognostic factors with positive impact on both OS (HR 3.051, 95% CI 0.982–9.476, $P = .054$ and HR 3.862, 95% CI 1.099–13.573, $P = .035$, respectively) and DSS (HR 3.751, 95% CI 1.045-13.466, P=.043 and HR 5.304, 95% CI 1.186–23.724, P=.029, respectively) (Table 3). Only IL-8 of circulating cytokines was a significant prognostic factor, where higher values of IL-8 had negative impact on OS and DSS (HR .263, 95% CI 0.085–.818, $P = .021$ and HR .133, 95% CI 0.3–.595, $P = .008$, respectively). Multivariate analysis indicated that level of IL-8 was the only significant prognostic factor for DSS in patients with advanced laryngeal cancer, where higher values had a negative impact on DSS. (Table 4).

Discussion

Tumor progression is partly based on various pathways of tumor immune evasion and suppression. Head and neck squamocellular carcinoma manifest this activity thorough

Table 2. Correlation between demographic and clinical parameters and TILs, circulatory cytokines and CRP levels.

	CD4		CD8		IL-6		IL-8		CRP	
	Correlation coefficient (N=52)	*P* value	Correlation coefficient (N=52)	*P* value	Correlation coefficient (N=52)	*P* value	Correlation coefficient (N=52)	*P* value	Correlation coefficient (N=52)	*P*value
Age	−.89	.532	−.85	.55	.53	.711	.145	.307	.309	.026
Histological grade	−.107	.449	0.2	.887	0.2	.887	.036	.802	−.215	.126
T Stage	−.185	.189	−.178	.206	.208	.139	.208	.139	.047	.738
N stage	−.182	.197	−.324	.019	.003	.981	.188	.182	.117	.41
Treatment	−.045	.75	−.038	.79	.196	.164	.196	.164	.108	.445

IL-6- Interleukin 6, IL-8- Interleukin 8, CRP-C reactive protein.

reducing their innate immunogenicity and suppressing the signals of antitumor immune response. Tumor immune microenvironment (TIME) is formed during tumor progression. It consists of tumor cells, immune cells, and their characteristic inflammatory response.[16] A major factor of TIME in determining tumor progression are tumor-infiltrating lymphocytes (TIL), as well as overall proportion and character of T cells within the TIME.[17] Previous studies showed a prognostic favorable role for several subtypes of tumor-infiltrating T cells, especially CD4 and CD8.[5,6,18] In our study, higher expression of $CD4^+$ and $CD8^+$ TILs were significant prognostic factors with positive impact on both OS and DSS in univariate analysis, but not in multivariate analysis. High tumoral and peritumoral density of CD4 and CD8 was identified as a positive predictor of OS, DSS, and disease free survival (DFS) in other studies as well.[19-21]

It was established that patients with laryngeal cancer and higher expression of TIL have higher sensitivity to chemoradiotherapy and higher survival probability. On the other hand, CD8 was not marked as significant prognostic marker in patients with surgically treated laryngeal carcinoma.[5,22,23] Our cohort of patients primarily involved surgically treated patients with postoperative radiotherapy or chemoradiotherapy. This could be the reason that higher expression of TILs did not prove to be a significant predictor of OS and DSS according to multivariate analysis.

IL-6 and IL-8 are classified as pro-inflammatory cytokines that have an important role in cancer angiogenesis, cancer cell growth, migration, and invasion and metastasis.[24-27] Significantly higher levels of IL-6 comparing to healthy subjects were established in patients with laryngeal cancer.[28] It appeared that higher levels of this cytokine directly correlated with the locoregional spread and the recurrence of the disease.[29-32]

In the study conducted by Hao et al on 92 patients with both early and advanced stages of laryngeal cancer, elevated levels of IL-6 were significant independent predicting factor of disease progression and overall survival. IL-8 was not labeled as significant predicting factor.[10] Our study identified levels of IL-8 as an individual significant predictor of 3-year OS and DSS survival in patients with advanced laryngeal cancer, but not levels of IL-6. IL-8 was identified as angiogenesis stimulator in cancer progression, which resulted in enhanced tumor growth and worse survival. Overexpression of IL-8 was also associated with a drug-resistant phenotype and decreased sensitivity to chemotherapy.[33] Our result could not be explained, other than that the study included only patients with advanced laryngeal cancer, where others included patients with all stages of the disease. Current knowledge on the role of cytokines in prognosis of laryngeal cancer is still limited, and more research is needed to understand how they could be used in prevention and treatment of the disease.

There have been numerous studies implicating CRP as a possible prognostic factor in solid tumors.[12,34–38] CRP is a non-specific marker of acute phase inflammatory response. It is synthesized primarily in liver hepatocytes and stimulated by pro-inflammatory cytokines (IL-6, IL-1, and tumor necrosis alpha).[39] The CRP level in normal human serum ranges from .2 to 10 mg/L. About 90% of apparently healthy individuals have CRP levels <3 mg/L.[40] CRP levels are elevated in response to injury, infection, and inflammation, but there is a growing body of evidence that CRP also has a functional role in the inflammatory process by activating classical complement pathway, inducing phagocytosis, chemotaxis of circulating leukocytes to areas of inflammation, and influencing apoptosis.[41] Previous data regarding CRP as a prognostic factor of survival in laryngeal cancer are scarce and inconsistent. Few studies confirmed that higher values of CRP were significantly correlated with malignant laryngeal disease and were in fact predictors of worse OS in patients with laryngeal cancer.[42-45] In our study CRP values were not predictive of OS and DSS in the 3-year follow-up period.

Main limitation of the study is limited number of included patients. Also, preferred treatment of laryngeal carcinoma in our referral tertiary center is surgery, followed by radio- or chemotherapy. Larger cohorts of patients involving randomized clinical trials involving larger patient cohorts would be a step further in establishing true use of these biomarkers in diagnostics, prognosis, and systemic treatment of laryngeal carcinoma.

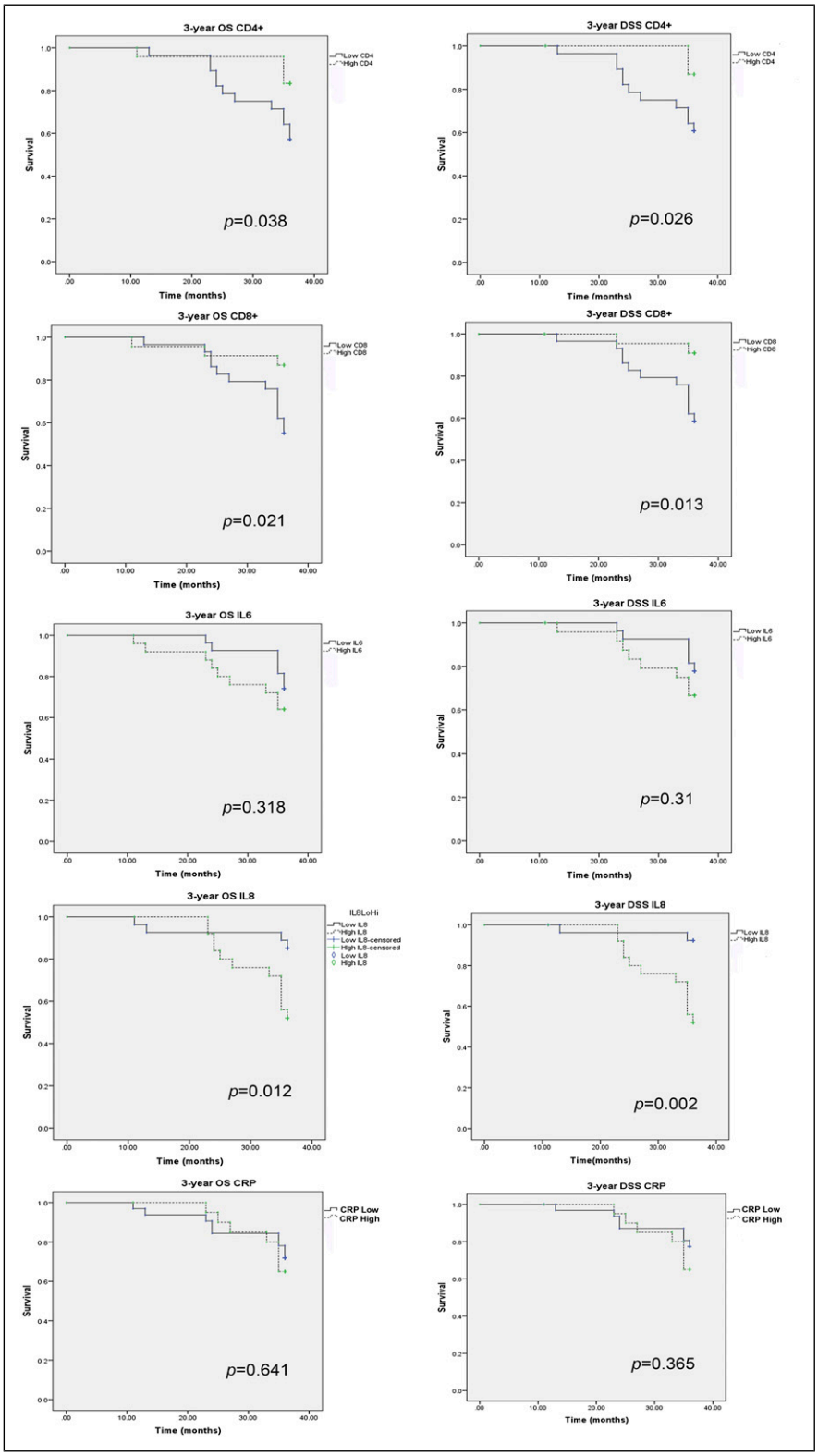

Figure 1. Kaplan–Meier curves for OS and DSS depending on TILs and levels of cytokines and CRP.

Table 3. Univariate Cox regression analyses for DFS and OS for 3-year follow-up period.

	OS				DSS			
	B	HR	95% CI	*p*	B	HR	95% CI	*p*
CD4	1.116	3.051	.982-9.476	.054	1.322	3.751	1.045-13.466	.043
CD8	1.351	3.862	1.099-13.573	.035	1.668	5.304	1.186-23.724	.029
IL-6	−.489	.613	.228-1.648	.332	−0-531	.588	.204-1.695	.326
IL-8	−1.335	.263	.085-.818	.021	−2.019	.133	.3-.595	.008
CRP	−.23	.795	.296-2.135	.648	−.47	.625	.219-1.783	.379

OS- overall survival, DSS-disease specific survival, IL-6- Interleukin 6, IL-8- Interleukin 8, CRP-C reactive protein, HR – hazard ratio; CI – confidence interval.

Table 4. Multivariate Cox regression analyses for DFS and OS for the 3-year follow-up period.

	OS				DSS			
	B	HR	95% CI	*p*	B	HR	95% CI	*p*
CD4	.09	.914	.152-5.475	.250	0.215	.807	.104-6.257	.837
CD8	1.084	2.958	.466-18.786	.922	1.290	3.632	.383-34.416	.261
IL-6	−.343	.709	.23-2.189	.550	−.284	.753	.224-2.531	.647
IL-8	−1.041	.535	.098-1.271	.111	−1.702	.182	.35-.943	.042
CRP	−.346	1.413	.471-4.236	.537	−.249	1.283	.397-4.145	.677

OS- overall survival, DSS-disease specific survival, IL-6- Interleukin 6, IL-8- Interleukin 8, CRP-C reactive protein, HR – hazard ratio; CI – confidence interval.

Conclusion

The study demonstrated that high expression of CD4 and CD8, with lower values of IL-8 were significant positive predictor of OS in patients with advanced laryngeal carcinoma, but only in unilateral analysis. Higher values of IL-8 proved to be significant negative predictor of DSS. These findings could further collaborate the inclusion of combination of biomarkers in assessment of favorable treatment choice in patients with advanced laryngeal carcinoma.

Declaration of Conflicting Interests

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021;71(3): 209-249.
2. Nocini R, Molteni G, Mattiuzzi C, Lippi G, Service of Clinical Governance, Provincial Agency for Social and Sanitary Services, Trento 38123, Italy, Section of Clinical Biochemistry, Department of Neuroscience, Biomedicine and Movement, University of Verona, Verona 37134, Italy. Updates on larynx cancer epidemiology. *Chin J Cancer Res*. 2020;32(1):18-25.
3. SEER*Explorer: An interactive website for SEER cancer statistics [Internet]. Surveillance Research Program, National Cancer Institute. [Cited 2022. April 27]. Available from https://seer.cancer.gov/explorer/.
4. Bonomi M, Patsias A, Posner M, Sikora A. The role of inflammation in head and neck cancer. *Adv Exp Med Biol*. 2014; 816:107-127.
5. Borsetto D, Tomasoni M, Payne K, et al. Prognostic Significance of CD4+ and CD8+ Tumor-Infiltrating Lymphocytes in Head and Neck Squamous Cell Carcinoma: A Meta-Analysis. *Cancers*. 2021;13(4):781.
6. Hoesli R, Birkeland AC, Rosko AJ, et al. Proportion of CD4 and CD8 tumor infiltrating lymphocytes predicts survival in persistent/recurrent laryngeal squamous cell carcinoma. *Oral Oncol*. 2018;77:83-89.
7. Mann JE, Smith JD, Birkeland AC, et al. Analysis of tumor-infiltrating CD103 resident memory T-cell content in recurrent laryngeal squamous cell carcinoma. *Cancer Immunol Immunother*. 2019;68:213-222.
8. Andersson BÅ, Lewin F, Lundgren J, et al. Plasma tumor necrosis factor-α and C-reactive protein a biomarker for survival in

head and neck squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2014;140(3):515-519.
9. Uz U, Eskiizmir G. Association Between Interleukin-6 and Head and Neck Squamous Cell Carcinoma: A Systematic Review. *Clin Exp Otorhinolaryngol*. 2021;14(1):50-60.
10. Hao W, Zhu Y, Zhou H. Prognostic value of interleukin-6 and interleukin-8 in laryngeal squamous cell cancer. *Med Oncol*. 2013;30(1):333.
11. Astradsson T, Sellberg F, Berglund D, Ehrsson YT, Laurell GFE. Systemic Inflammatory Reaction in Patients With Head and Neck Cancer-An Explorative Study. *Front Oncol*. 2019;59: 1177.
12. Shrotriya S, Walsh D, Nowacki AS, et al. Serum C-reactive protein is an important and powerful prognostic biomarker in most adult solid tumors. *PLoS One*. 2018;13(8):e0202555.
13. Wang J, Wang S, Song X, et al. The prognostic value of systemic and local inflammation in patients with laryngeal squamous cell carcinoma. *OncoTargets Ther*. 2016;9:7177-7185.
14. Adelstein D, Gillison ML, Pfister DG, et al. NCCN Guidelines Insights: Head and Neck Cancers, Version 2.2017. *J Natl Compr Cancer Netw*. 2017;15(6):761-770.
15. Sher DJ, Adelstein DJ, Bajaj GK, et al. Radiation therapy for oropharyngeal squamous cell carcinoma: Executive summary of an ASTRO Evidence- Based Clinical Practice Guideline. *Pract Radiat Oncol*. 2017;7(4):246–53-253.
16. Ferris RL. Immunology and Immunotherapy of Head and Neck Cancer. *J Clin Oncol*. 2015;33(29):3293-3304. doi:10.1200/JCO.2015.61.1509.
17. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5):541-550.
18. Rodrigo JP, Sánchez-Canteli M, López F, et al. Tumor-Infiltrating Lymphocytes in the Tumor Microenvironment of Laryngeal Squamous Cell Carcinoma: Systematic Review and Meta-Analysis. *Biomedicines*. 2021;9(5):486.
19. Sanchez-Canteli M, Granda-Díaz R, Del Rio-Ibisate N, et al. PD-L1 expression correlates with tumor-infiltrating lymphocytes and better prognosis in patients with HPV-negative head and neck squamous cell carcinomas. *Cancer Immunol Immunother*. 2020;69(10):2089-2100.
20. Zhou L, Li Y, Gao W, et al. Assessment of tumor-associated immune cells in laryngeal squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2019;145(7):1761-1772.
21. Zhang D, Tang D, Heng Y, et al. Prognostic Impact of Tumor-Infiltrating Lymphocytes in Laryngeal Squamous Cell Carcinoma Patients. *Laryngoscope*. 2021;131(4):E1249-E1255.
22. Suzuki H, Kawasaki Y, Miura M, et al. Tumor Infiltrating Lymphocytes are Prognostic Factors and Can Be Markers of Sensitivity to Chemoradiotherapy in Head and Neck Squamous Cell Carcinoma. *Asian Pac J Cancer Prev APJCP*. 2022;23(4): 1271-1278.
23. Chatzopoulos K, Kotoula V, Manoussou K, et al. Tumor Infiltrating Lymphocytes and CD8+ T Cell Subsets as Prognostic Markers in Patients with Surgically Treated Laryngeal Squamous Cell Carcinoma. *Head Neck Pathol*. 2020;14(3):689-700.
24. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. *Tumour Biol*. 2016;37(9):11553-11572.
25. Gopinathan G, Milagre C, Pearce OM, et al. Interleukin-6 stimulates defective angiogenesis. *Cancer Res*. 2015;75(15): 3098-3107.
26. Alfaro C, Sanmamed MF, Rodriguez-Ruiz ME, et al. Interleukin-8 in cancer pathogenesis, treatment and follow-up. *Cancer Treat Rev*. 2017;60:24-31.
27. Li KC, Huang YH, Ho CY, et al. The role of IL-8 in the SDF-1α/CXCR4-induced angiogenesis of laryngeal and hypopharyngeal squamous cell carcinoma. *Oral Oncol*. 2012;48(6):507-515.
28. Nikakhlagh S, Ranjbari N, Khorami E, Saki N. Association between Serum Levels of Interleukin-6 and Stage of Laryngeal Cancer. *Iran J Otorhinolaryngol*. 2015;27(80):199-205.
29. Riedel F, Zaiss I, Herzog D, et al. Serum levels of interleukin-6 in patients with primary head and neck squamous cell carcinoma. *Anticancer Res*. 2005;25(4):2761-2765.
30. Duffy S, Taylor J, Terrel J, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *American Cancer Society*. 2008;113(4):750-757.
31. Sotirović J, Perić A, Vojvodić D, et al. Serum cytokine profile of laryngeal squamous cell carcinoma patients. *J Laryngol Otol*. 2017;131(5):455-461.
32. Cottin SC, Turcotte S, Douville P, Meyer F, Bairati I. Predictors of circulating interleukin-6 levels in head and neck cancer patients. *Cancers Head Neck*. 2018;3:1.
33. Ralli M, Grasso M, Gilardi A, et al. The role of cytokines in head and neck squamous cell carcinoma: A review. *Clin Ter*. 2020; 171(3):e268-e274.
34. Wang Y, Zhang Z, Wang J, Zhang X. Association between C-reactive protein level and subsequent risk of ovarian cancer: A meta-analysis of 13 cohorts in 1, 852 ovarian cancer patients. *Medicine (Baltim)*. 2020;99(5):e1882.
35. Liu T, Zhang Q, Song C, et al. C-reactive protein trajectories and the risk of all cancer types: A prospective cohort study. *Int J Cancer*. 2022;151:297-307. Epub ahead of print.
36. Ji M, Du L, Ma Z, et al. Circulating C-reactive protein increases lung cancer risk: Results from a prospective cohort of UK Biobank. *Int J Cancer*. 2022;150(1):47-55.
37. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-Reactive Protein Is Associated With Incident Cancer and Survival in Patients With Cancer. *J Clin Oncol*. 2009;2713:2217-2224.
38. Heikkilä K, Ebrahim S, Lawlor DA. A systematic review of the association between circulating concentrations of C reactive protein and cancer. *J Epidemiol Community Health*. 2007;61(9): 824-833.
39. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, inetleukin-6 and tumour necrosis factor-α. *Mol Psychiatr*. 2016;21:642-649.
40. Pagana KD, Pagana TJ, Pagana TN. *Mosby's Diagnostic & Laboratory Test Reference*. 14th ed. Elsevier; 2019.
41. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol*. 2018;9:754.

42. Şahİn B, Akyol M, Özel HE, GenC S. Correlation of Clinical and Pathological Diagnosis and Inflammatory Markers for Patients Undergoing Laryngeal Microsurgery. *J Voice*. 2020; S0892-1997(20):30377.
43. Zeng YC, Xue M, Chi F, et al. C-reactive protein level predicts prognosis in patients with locoregionally advanced laryngeal carcinoma treated with chemoradiotherapy. *Tumour Biol*. 2012; 33(3):891-895.
44. Fu J, Yang X. The Prognostic Value of the C-reactive Protein/ Prognostic Nutritional Index Ratio in Stage III and IV Laryngeal Cancer Patients Treated with Radiotherapy. *Cureus*. 2019;11(5): e4648.
45. Chen HH, Wang HM, Fan KH, et al. Pre-treatment levels of C-reactive protein and squamous cell carcinoma antigen for predicting the aggressiveness of pharyngolaryngeal carcinoma. *PLoS One*. 2013;8(1):e55327.