Infiltration of CD1a-Positive Dendritic Cells in Advanced Laryngeal Cancer Correlates with Unfavorable Outcomes Post-Laryngectomy

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Research article

Keywords: laryngeal cancer, CD1a, dendritic cell, prognosis, immunohistochemistry

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-60845/v1

Abstract

Background: The prognosis of advanced laryngeal cancer is unfavorable despite the progress of multidisciplinary therapy. Dendritic cells (DCs) play a central role in antitumor immunity. Tumor-infiltrating $CD1a^+$ DCs have been reported to be associated with clinical outcomes in carcinomas of various organs, but the clinical impact of $CD1a^+$ DCs in laryngeal cancer has not been clear.

Patients and Methods: We retrospectively analyzed the cases of 57 patients with Stage □ or □ laryngeal cancer who underwent a total laryngectomy. Immunohistochemistry for CD1a, S100, and CD8 was performed using representative resected specimens. $CD1a^+$ DCs, $S100^+$ DCs, and $CD8^+$ cytotoxic T-lymphocytes (CTLs) were evaluated, and we divided the cases into high and low groups by the cut-off of the median value for each of these three parameters.

Results: Compared to the CD1a-low group, the CD1a-high group had more advanced cases and showed significantly worse disease-specific survival (DSS) (p=0.0082) and overall survival (OS) (p=0.0324). The analyses of S100 DCs and $CD8^+$ CTLs revealed no significant impact on clinical outcomes. Multivariate analyses indicated that the infiltration of $CD1a^+$ DCs is an independent unfavorable prognostic factor in both DSS (p=0.0093) and OS (p=0.0132).

Conclusions: Our results demonstrated that the infiltration of $CD1a^+$ DCs was associated with unfavorable clinical outcomes. It is not yet known why the presence of $CD1a^+$ DCs lead to an unfavorable clinical outcome in laryngeal cancer. Further studies are necessary to validate our results and clarify the function(s) of tumor-infiltrating $CD1a^+$ DCs.

Introduction

Laryngeal cancer is the second most common malignancy in head and neck cancer, following thyroid cancer [1]. Although laryngeal cancer accounts for only 0.9% for men and 0.2% for women among all cancers, approx. 4,800 patients are newly diagnosed with laryngeal cancer per year in Japan [2]. The 5-year survival rate of early laryngeal cancer (Stages □ and □ in the TNM classification) is favorable (> 80%), but the prognosis of advanced laryngeal cancer (Stages III and IV in the TNM classification) is poor, and its 5-year survival rate remains around 50% despite the progress of multidisciplinary therapy such as combinations of chemo-radiotherapy and surgery [3]. The establishment of distinct prognostic factors and the development of novel treatments for advanced laryngeal cancer are therefore important for the proper assessments of prognoses and for decisions about the appropriate therapeutic strategy.

Cancer immunity plays an important role in the suppression of the invasion and proliferation of solid tumors, but some types of cancer cells develop immune tolerance and can escape the T-cell mediated antitumor immune response [4, 5]. It is thus necessary to determine the precise status of the immune response in patients with advanced laryngeal cancer; doing so is likely to contribute to the development of distinct prognostic factors and/or novel treatments. In the present study, we focused on the dendritic

cells (DCs) in laryngeal cancer, which are generally considered central regulators of anticancer immune responses [6]. DCs are representative antigen-presenting cells that activate cytotoxic T-lymphocytes (CTLs) via major histocompatibility complex class I and class II molecules [7]. Dendritic cells also directly activate B lymphocytes [8] and can activate innate immune cells such as natural killer cells and natural killer T cells [9–11]. Thus, DCs are considered to play a central role in antitumor immunity.

CD1a is a transmembrane glycoprotein that is associated with the antigen presentation of DCs. In contrast to the S100 protein, which is usually expressed on both immature and mature DCs [12], CD1a is generally considered to be specifically expressed on immature DCs [13]. Several reports have indicated that the infiltration of CD1a-positive ($CD1a^+$) DCs into tumor tissue is associated with favorable clinical outcomes in carcinomas of the ovary [14], oral cavity [15], and thyroid [16]. However, the roles of tumor-infiltrating $CD1a^+$ DCs and their clinical impact on patients with laryngeal cancer are not yet known. We conducted the present study to (1) assess the status of tumor-infiltrating $CD1a^+$ DCs in patients with advanced laryngeal cancer and (2) clarify the relationships between $CD1a^+$ DCs and clinicopathological characteristics including outcomes.

Patients And Methods

Patients

The initial enrollees were 656 patients with laryngeal cancer who were treated at Saga University Hospital between 1990 and 2016. Among them, we excluded neoadjuvant cases including those treated with chemotherapy with or without radiotherapy. The 93 patients who underwent a total laryngectomy as the initial treatment for laryngeal cancer were assessed; there were 33 cases at Stage □, 22 cases at Stage □, and 38 cases at Stage □ based on the TNM classification (8th ed.) [17]. We excluded the Stage □ cases because none of those patients died of cancer progression. Of the remaining 60 cases with Stage □ and □ laryngeal cancers, three cases were excluded because of the unavailability of cancer tissue. The final total of 57 total laryngectomy cases with Stage □ or □ laryngeal cancer was retrospectively analyzed. Comprehensive informed consent for the use of resected tissue for research was obtained from all patients, and the study protocol was approved by the Ethics Committee of the Faculty of Medicine at Saga University (No. 2020-04-R-19).

Immunohistochemistry

The 4-μm cut sections of formalin-fixed paraffin-embedded specimens of representative cancer tissue from each patient's case were used for immunohistochemistry (IHC). The primary antibodies used were CD1a (Clone010, Ready to use; Dako, Glostrup, Denmark), and CD8 (CloneC8/144B, Ready to use; Dako). IHC was performed on using an Autostainer plus® automatic stainer (Dako). The Envision+® System (Dako) was used as the secondary antibody. Slides were visualized by diaminobenzidine tetrahydrochloride, and nuclei were counterstained with hematoxylin.

Assessment of DCs and CD8$^+$ CTLs

For the evaluation of CD1a$^+$ and S100$^+$ DCs, we took digital photographs of three hot spots of DC infiltration of tumor tissue on a light microscope (×100) for each patient's case. The number of DCs was counted in each digital photograph, and the average number of the three digital images was calculated in each case. For the evaluation of CD8$^+$ CTLs, we took digital photographs of three hot spots of CTL infiltration of tumor tissue on a light microscope (×200). The average numbers of tumor-infiltrating CD8$^+$ CTLs in three digital images were automatically calculated by image analysis software (Tissue Studio, Definiens, Munchen, Germany). Representative analyzed images are demonstrated in **Figure 1**. We divided the patients into pairs of groups based the median value in each assessment of CD1a$^+$, S100$^+$, and CD8$^+$ cells.

Statistical analyses

All statistical analyzes were performed using JMP Pro 13.1.0 software (SAS Institute, Cary, NC). Student's t-test, Pearson's chi-square test, and a linear regression analysis were used as appropriate for comparisons between two groups. Disease-specific survival (DSS) was defined as the period from surgery to cancer-related death or the last follow-up, and overall survival (OS) was defined as the period from surgery to death or last follow-up. The maximum follow-up period for this study was 120 months, with a median follow-up of 45.2 months. The survival curve was calculated by the Kaplan-Meier method, and a Log-rank test was also performed. Univariate and multivariate analyses were performed using Cox's proportional hazards model. Significant variables in the univariate analyses were selected for the multivariate analysis. Probability (p)-values <0.05 were considered significant.

Results

Clinicopathologic features of the 57 patients with advanced laryngeal cancer

The clinicopathologic features of the 57 patients with advanced laryngeal cancer are summarized in **Table 1**. Fifty-four patients (94.7%) were male and the remaining three patients (5.3%) were female. The mean age at the time of surgery was 68.4 years. The most frequent primary tumor site was the glottis (n=35), followed by the supraglottis (n=16) and subglottis (n=6). Regarding the T stage, four cases (7.0%) were categorized as T2, 22 cases (38.6%) were categorized as T3, and 31 cases (54.4%) were categorized as T4. Thirty-seven cases (64.9%) had no lymph-node metastasis at the time of surgery. Only one patient had distant metastasis. Twenty cases (35.1%) were categorized as Stage III, and the remaining 37 cases (64.9%) are categorized as Stage □. The histology of all 57 cases was squamous cell carcinoma (SCC), and its histological differentiation was distributed as follows: well-differentiated SCC (n=30), moderately differentiated SCC (n=25), and poorly differentiated SCC (n=2). Thirty-eight patients (66.6%) received adjuvant therapy; the details are as follows: 32 patients received chemotherapy (uracil/tegafur [n=29], tegafur [n=2], cisplatin+5-FU [n=1]), two patients received radiotherapy, and four patients received

chemoradiotherapy; the chemotherapy regimens were: uracil/tegafur (n=1), TS-1 (n=1), cisplatin (n=1), nedaplatin (n=1).

Assessment of CD1a$^+$ DCs and theircorrelations with clinicopathologic factors

An infiltration of CD1a$^+$ DCs was observed in all 57 cases with various densities in areas adhering to or adjacent to the tumor cells. The average number of tumor-infiltrating CD1a$^+$ DCs was 46.92 (range 1.3–351). Using the median value (28.7) of the number of infiltrating CD1a$^+$ DCs as a cut-off, we divided the cases into the CD1a-low group (n=29) and the CD1a-high group (n=28). Representative histological images from each group are presented in **Figure 2**.

The relationships between CD1a$^+$ DC infiltration and clinicopathologic factors are summarized in **Table 2**. The CD1a-high group had significantly more advanced (T4 and Stage) cases (p=0.0448 and p=0.0377, respectively) than the CD1a-low group. No significant difference was observed between the CD1a-low and -high groups in other factors, i.e., age, gender, smoking, alcohol consumption, primary site, histological differentiation, T stage, N stage, M stage, or adjuvant therapy.

Assessment of S100$^+$ DCs and their correlations with clinicopathologic factors

Infiltration of S100$^+$ DCs was also observed in all 57 cases with various densities. The average number of tumor-infiltrating S100$^+$ DCs was 68.9 (range 0.5–390.3). Using the median value (49.3) of S100$^+$ DCs as a cut-off, we divided the cases into the S100-low group (n=29) and the S100-high group (n=28). Representative histological images from each group are provided in **Figure 3**. The relationships between S100$^+$ DC infiltration and clinicopathologic factors are summarized in **Table 3**. Compared to the S100-low group, the S100-high group had significantly more cases of well-differentiated SCC (p=0.0120). No significant difference was observed in other factors (age, gender, smoking, alcohol drinking, primary site, T stage, N stage, M stage, or adjuvant therapy) between the S100-low and S100-high groups.

Assessment of CD8$^+$ CTLs and their association with CD1a$^+$ and S100$^+$ DCs

The average number of tumor-infiltrating CD8$^+$ CTLs was 562.8 (range 0–2,580). The results of the linear regression analysis between CD1a$^+$ DCs or S100$^+$ DCs and CD8$^+$ CTLs are illustrated in **Figure 4**. The cases with a higher number of tumor-infiltrating CD1a$^+$ DCs tended to have a higher number of CD8$^+$ CTLs, although no significance was observed (p=0.4278). Similarly, the cases with a higher number of tumor-infiltrating S100$^+$ DCs tended to have a higher number of CD8$^+$ CTLs; no significance was observed (p=0.5409). We divided the cases into the CD8-low group (n=28) and CD8-high group (n=29) using the median value of CD8$^+$ CTLs (435) as a cut-off value. No significant difference in clinicopathologic factors was observed between these groups.

Kaplan-Meier survival curves according to the infiltration of DCs and CD8$^+$ CTLs

The Kaplan-Meier survival curves based on the status of $CD1a^+$ DCs, $S100^+$ DCs, and $CD8^+$ CTLs are shown in **Figure 5.** The CD1a-low group showed significantly better DSS and OS than the CD1a-high group (p=0.0082 and 0.0324, respectively). No significant difference was observed between the S100-low group and S100-high group (p=0.3098 and p=0.5106) or between the CD8-low group and CD8-high group (p=0.2575 and p=0.5045) in both DSS and OS.

Univariate and multivariate analyses for OS and DSS

The results of the univariate analyses for OS and DSS are summarized in **Table 4**. The factors significantly correlated with OS were tumor subsite, T stage, N stage, and infiltration of $CD1a^+$ DCs (p=0.0457, p=0.0083, p=0.0027, p=0.0351, respectively). The factors significantly correlated with DSS were T stage, N stage, and infiltration of $CD1a^+$ DCs (p=0.0002, p=0.0240, p=0.0089, respectively).

The factors that were shown to be significant in the univariate analyses were further subjected to the multivariate analyses (**Table 5**). The multivariate analysis for OS indicated that T stage, N stage, and infiltration of $CD1a^+$ DCs were each significantly associated with the patients' OS (p=0.0256, p=0.0069, p=0.0132 respectively). The multivariate analysis for DSS indicated that T stage, N stage, and infiltration of $CD1a^+$ DCs were significantly associated with the patients' DSS (p=0.0005, p=0.0209, and p=0.0093 respectively).

Discussion

We investigated the infiltration of $CD1a^+$ DCs and its association with clinicopathological factors in patients with advanced laryngeal cancer who underwent a total laryngectomy as an initial treatment. Unexpectedly, the CD1a-high group showed unfavorable clinical outcomes, whereas tumor-infiltrating $S100^+$ DCs were not significantly associated with clinical outcomes.

It has been speculated that a higher density of $CD1a^+$ DCs in tumor tissue correlates with favorable clinical outcomes, and several studies indicated that tumor-infiltrating $CD1a^+$ DCs were associated with favorable clinical outcomes in carcinomas of various organs [14–16]. However, conflicting results have also been reported. Hilly et al. [18] reported that higher $CD1a^+$ DCs infiltration around the tumor was associated with a higher risk of recurrence in surgically treated cases of early squamous cell carcinoma of the tongue. Lundgren et al. [19] reported that a high density of infiltrating $CD1a^+$ DCs is an unfavorable prognostic factor in the pancreato-biliary type of periampullary adenocarcinoma. One of the possible reasons for this discrepancy in findings is the widely varying methods used to evaluate $CD1a^+$ DCs in the previous series. Taken together, the past and present findings show that the relationship between tumor-infiltrating $CD1a^+$ DCs and clinical outcomes in patients with malignancies remains controversial.

Our literature search revealed four studies investigating tumor-infiltrating DCs in laryngeal cancer, and three of these four studies investigated $S100^+$ DCs only. Yilmaz et al. [20] and Gallo et al. [21] reported that a high density of tumor-infiltrating $S100^+$ DCs is associated with favorable outcomes. However,

Karakök et al. reported that the infiltration of $S100^+$ DCs was not associated with survival, although it was significantly associated with the inflammatory response [22]. This result is consistent with our analysis of $S100^+$ DCs. Only one of these four previous studies investigated $CD1a^+$ DC infiltration in laryngeal cancer. Esteban et al. focused on CD1a ($OKT6^+$) DCs and reported that the infiltration of $CD1a^+$ DCs was not associated with survival, although it was significantly associated with lymphocyte infiltration [23]. Thus, the present study is the first to report an association between poor surgical outcomes and tumor-infiltrating $CD1a^+$ DCs in laryngeal cancer.

It is generally considered that immature DCs capture the tumor antigen and then mature and present the antigen to naïve T cells, which then induces a cellular immune response involving $CD8^+$ CTLs [24]. In the present study, the number of $CD8^+$ CTLs was higher in the $CD1a^+$ DC-high group, although not significantly. A possible explanation of the correlation of the adverse effect of $CD1a^+$ DCs is that $CD1a^+$ DCs have a specific but unknown function other than antigen presentation that accelerates the progression of cancer cells. It can also be hypothesized that the function of $CD1a^+$ DCs in tumor tissue may differ according to the organs or histological types. As it is known that there are many subsets of DCs with unique and specific functions [25], these hypotheses seem plausible. However, the role(s) of $CD1a^+$ DCs in cancer tissue remain unclear, as does the reasons why the invasion of $CD1a^+$ DCs correlates with a poor prognosis of patients with advanced laryngeal cancer.

It has been reported that head and neck SCC reduces the body's immunocompetence in multiple ways [26]. A malfunction or decrease of plasmacytoid dendritic cells (pDCs) in tumor tissue is considered one of the causes of reduced immunocompetence because pDCs produce interferon (IFN), which plays an important role in antitumor immunity [27]. However, on the contrary, without stimulation (e.g., by viruses), T cell derived CD40 ligand activates pDCs, and these activated pDCs support the functions of regulatory T cells and contribute to immunotolerance [28, 29]. O'Donnell et al. reported that an intratumoral increase of Langerin-positive immature DCs was significantly associated with vascular/lymphatic invasion and unfavorable survival in patients with oral SCC. They also demonstrated that the presence of CD123-positive pDCs was associated with a poor prognosis [30]. Thus, a malignancy's microenvironment that is induced by the immune system is extremely complex, and most of the functions and potential interactions among various subsets of DCs remain unknown.

The limitations of this study are its retrospective nature, the relatively small number of patients treated at a single center, and the long period required for enrollment. In addition, the immunohistochemical analyses was performed using only one representative section of cancer tissue, and therefore the entire tumor tissue was not evaluated.

In conclusion, we have analyzed the association between the infiltration of $CD1a^+$ DCs into cancer tissue and clinicopathologic factors in patients with advanced laryngeal cancer. Our results demonstrated that $CD1a^+$ DC infiltration was associated with poor clinical outcomes, and it was an independent prognostic factor in multivariate analyses. The mechanism of $CD1a^+$ DCs leading to unfavorable clinical outcomes

remains to be clarified. We hope that further studies will validate our results and elucidate the function(s) of tumor-infiltrating $CD1a^+$ DCs in advanced laryngeal cancer.

Abbreviations

CTL
cytotoxic T-lymphocyte, DC:dendritic cell, DSS:disease-specific survival, IHC:immunohistochemistry, OS:overall survival, SCC:squamous cell carcinoma

Declarations

Ethics approval and consent to participate

Comprehensive informed consent for the use of resected tissue for a research was obtained from all patients, and the study protocol was approved by the Ethics Committee of the Faculty of Medicine at Saga University (No. 2020-04-R-19).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the specifics of the patients' informed consent and because the study's ethics approval did not cover this issue, but they are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

Funding

There was no grant support for this work.

Authors' contributions

Conceptualization, project administration: Kai K. and Aishima S.; data curation and formal analysis: Minesaki A. and Kai K.; validation: Kuratomi Y. and Aishima S.; writing of the original draft: Minesaki A. and Kai K.; writing - review & editing: Kuratomi Y. and Aishima S.

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Tables

Table 1
Clinicopathologic features of the 57 patients with laryngeal cancer

Age, yrs; mean ± SD)		**68.4 ± 8.8**
Gender	Male	54 (94.7%)
	Female	3 (5.3%)
Smoking habit	Never	5 (8.8%)
	Ex	19 (33.3%)
	Current	33 (57.9%)
Alcohol abuse	(+)	35 (61.4%)
	(−)	22 (38.6%)
Subsite	Glottis	35 (61.4%)
	Supraglottis	16 (28.1%)
	Subglottis	6 (10.5%)
Histology*	Well	30 (52.6%)
	Mode	25 (43.9%)
	Poor	2 (3.5%)
T stage	T2	4 (7.0%)
	T3	22 (38.6%)
	T4	31 (54.4%)
N stage	N0	37 (64.9%)
	N1	5 (8.8%)
	N2	14 (24.6%)
	N3	1 (1.8%)
M stage	M0	56 (98.2%)
	M1	1 (1.8%)
Stage	□	20 (35.1%)
	□	37 (64.9%)
Adjuvant therapy	None	19 (33.3%)
	Radiotherapy	2 (3.5%)

Age, yrs; mean ± SD)		**68.4 ± 8.8**
	Chemotherapy	32 (56.1%)
	Chemoradiotherapy	4 (7.0%)
*The histology of all cases was squamous cell carcinoma.		

Table 2
Clinicopathologic features per CD1a+ DC infiltration

		CD1a Low (n = 29)	**CD1a High (n = 28)**	**p-value**
Age, yrs; mean ± SD		68.9 ± 8.4	67.9 ± 9.3	0.6823
Gender	Male	28 (96.6%)	26 (92.9%)	0.5323
	Female	1 (3.4%)	2 (7.1%)	
Subsite	Glottic	17 (58.6%)	18 (64.3%)	0.6605
	Supra/Sub	12 (41.4%)	10 (35.7%)	
Histology	Well	16 (55.2%)	14 (50.0%)	0.6958
	Mode/poor	13 (44.8%)	14 (50.0%)	
T stage	T2/T3	17 (58.6%)	9 (32.1%)	0.0448
	T4	12 (41.4%)	19 (67.9%)	
N stage	N0	20 (69.0%)	17 (60.7%)	0.5140
	N1-3	9 (31.0%)	11 (39.3%)	
M stage (%)	M0	28 (96.6%)	28 (100.0%)	0.3215
	M1	1 (3.4%)	0 (0.0%)	
Stage	□	14 (48.3%)	6 (21.4%)	0.0337
	□	15 (51.7%)	22 (78.6%)	
$CD8^+$ CTLs		510.7 ± 582.6	616.7 ± 415.6	0.4278
Adjuvant therapy	(−)	8 (27.6%)	11 (39.3%)	0.3489
	(+)	21 (72.4%)	17 (60.7%)	

Table 3
Clinicopathologic features per S100+ DC infiltration

		S100 Low (n = 29)	S100 High (n = 28)	p-value
Age, mean ± SD		68.4 ± 10.0	68.4 ± 7.6	0.9950
Gender (%)	Male	28 (96.6%)	26 (92.9%)	0.5323
	Female	1 (3.4%)	2 (7.1%)	
Subsite	Glottic	19 (65.5%)	16 (57.1%)	0.5162
	Supra/sub	10 (34.5%)	12 (42.9%)	
Histology	Well	20 (69.0%)	10 (35.7%)	0.0120
	Mode/poor	9 (31.0%)	18 (64.3%)	
T stage (%)	T2/T3	12 (41.4%)	14 (50.0%)	0.5136
	T4	17 (58.6%)	14 (50.0%)	
N stage (%)	N0	22 (75.9%)	15 (53.6%)	0.0779
	N1–3	7 (24.1%)	13 (46.4%)	
M stage (%)	M0	28 (96.6%)	28 (100.0%)	0.3215
	M1	1 (3.4%)	0 (0.0%)	
Stage	□	11 (37.9%)	9 (32.1%)	0.6471
	□	18 (62.1%)	19 (67.9%)	
$CD8^+$ CTLs		499.2 ± 513.6	628.6 ± 498.2	0.5409
Adjuvant therapy	(−)	11 (37.9%)	8 (28.6%)	0.4536
	(+)	18 (62.1%)	20 (71.4%)	

Table 4. Univariate analyses for disease specific-survival and overall survival (n=57)

Characteristic		n	DSS			OS	
			HR (95%CI)	p-value		HR (95%CI)	p-value
Age				0.919			0.7885
	≤67	28	1			1	
	67<	29	1.05 (0.40–2.73)			1.12 (0.49–2.55)	
Gender				0.3147			0.0810
	Female	3	1			1	
	Male	54	0.29 (0.04–2.25)			0.20 (0.05–0.89)	
Subsite				0.1490			0.0457
	Glottic	35	1			1	
	Supra/sub	22	2.02 (0.78–5.25)			2.31 (1.01–5.28)	
Histology				0.5749			0.9368
	Well	30	1			1	
	Mode/poor	27	0.76 (0.29–2.00)			1.03 (0.45–2.35)	
T stage				0.0002			0.0083
	T2/T3	26	1			1	
	T4	31	8.93 (2.04–39.18)			3.24 (1.27–8.25)	
N stage				0.0240			0.0027
	N0	37	1			1	
	N1-3	20	3.05 (1.17–7.93)			3.58 (1.56–8.19)	
M stage				0.4355			0.5300
	M0	56	1			1	
	M1	1	2.50 (0.33–19.01)			2.04 (0.27–15.32)	
Adjuvant therapy				0.2693			0.5706
	(−)	19	1			1	
	(+)	38	1.83 (0.59–5.61)			1.29 (0.53–3.14)	
$CD1a^+$ DCs				0.0089			0.0351
	Low	43	1			1	
	High	14	3.76 (1.31–10.74)			2.45 (1.05–5.70)	
$S100^+$ DCs				0.4161			0.6722
	Low	39	1			1	
	High	18	1.49 (0.57–3.92)			1.19 (0.52–2.72)	
$CD8^+$ CTLs				0.2534			0.5039
	Low	33	1			1	
	High	24	1.77 (0.65–4.84)			1.33 (0.58–3.06)	
DSS: disease-specific survival, OS: overall survival.							

Table 5. Multivariate analyses for disease specific- and overall survival (n=57)

Type	Characteristic	HR (95%CI)	p-value
DSS	CD1a (high)	4.03 (1.32–12.29)	0.0093
	T stage (T4)	8.48 (1.89–37.98)	0.0005
	N stage (positive)	3.21 (1.21–8.57)	0.0209
OS	CD1a (high)	3.14 (1.24–7.95)	0.0132
	Subtype (glottic)	0.47 (0.19–1.13)	0.0904
	T stage (T4)	2.78 (1.07–7.22)	0.0256
	N stage (positive)	3.23 (1.38–7.54)	0.0069
DSS: disease-specific survival, OS: overall survival.			

Figures

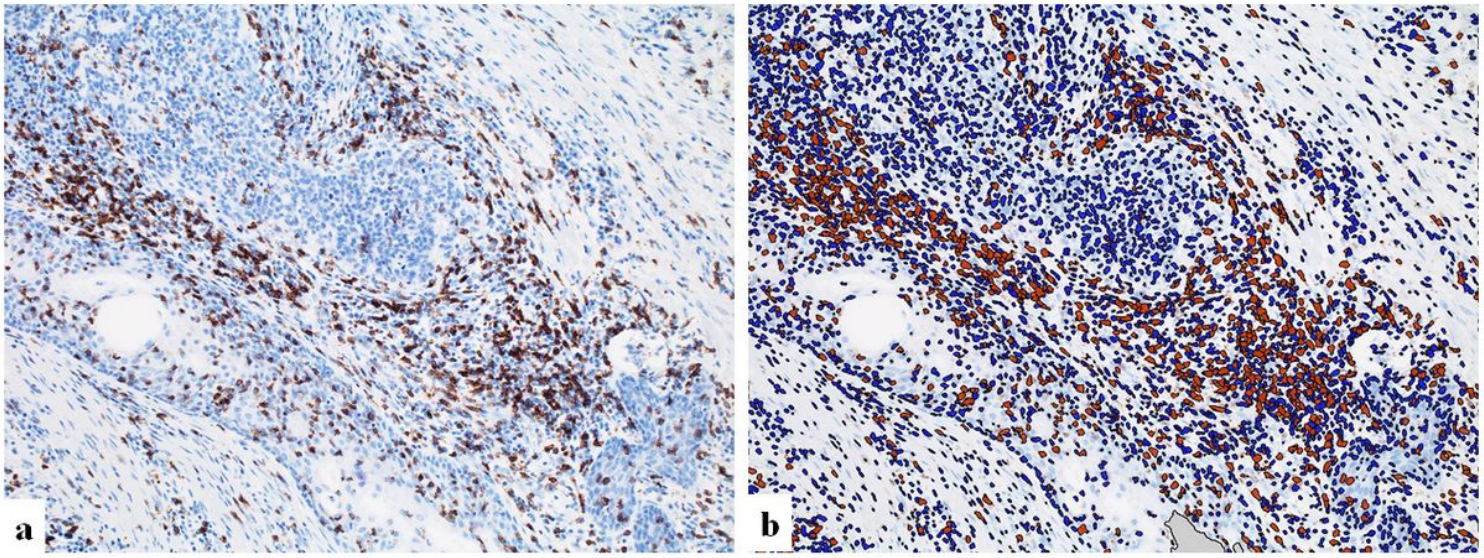

Figure 1

Representative analyzing images for CD8+ CTLs. a: Digital image of IHC for CD8 (×200). b: CD8+ CTLs automatically detected (orange) by the image analysis software (Tissue Studio).

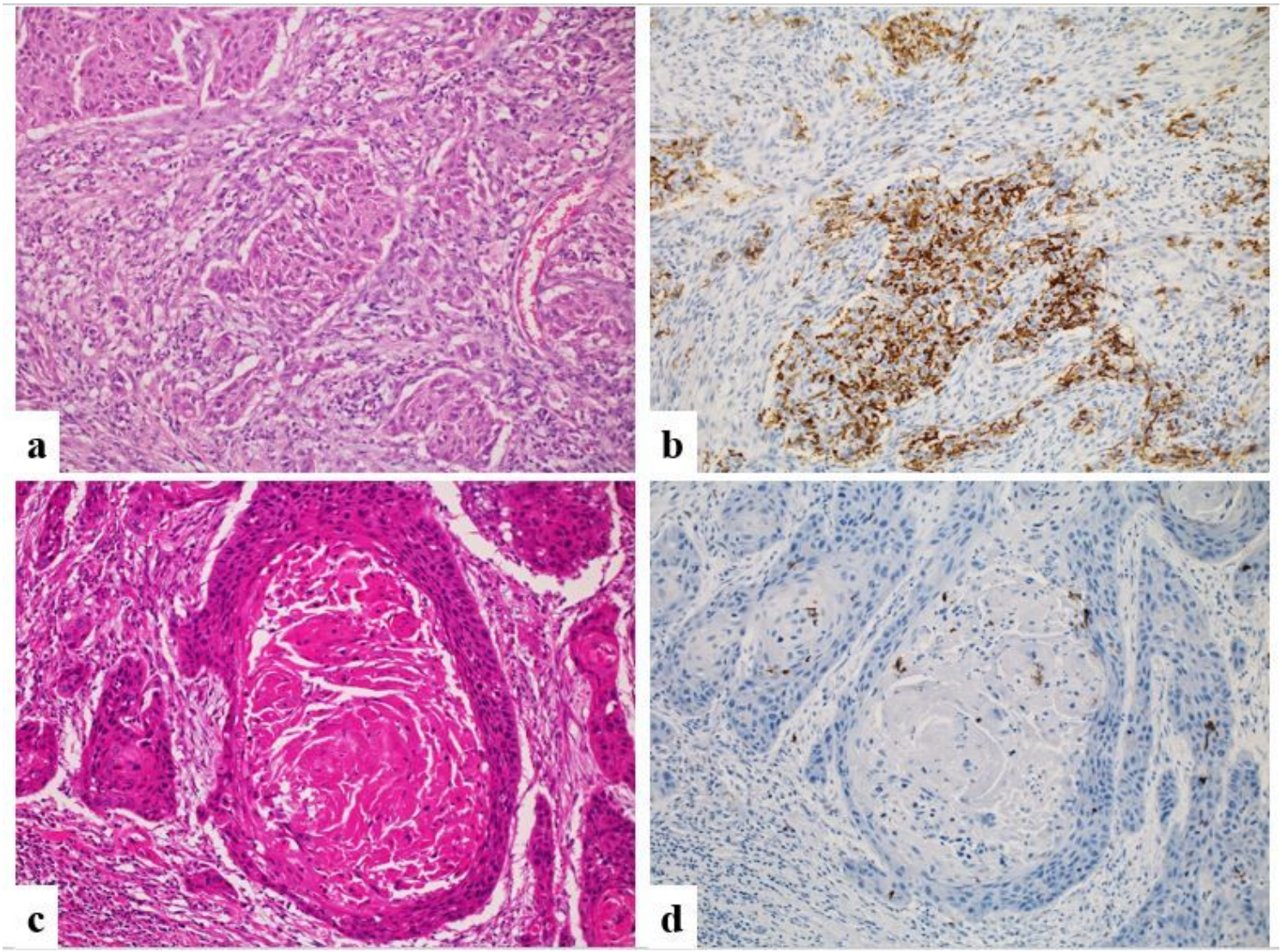

Figure 2

Representative histological images of CD1a+ DC infiltration into laryngeal squamous cell carcinoma. a: Hematoxylin and eosin (HE)-stained image of a CD1a-high case (×200). b: IHC of CD1a in a CD1a-high case (×200). Numerous CD1a+ DCs can be observed. c: IHC of a CD1a-low case (×200). d: IHC of CD1a in a CD1a-low case (×200). Few CD1a+ DCs are present.

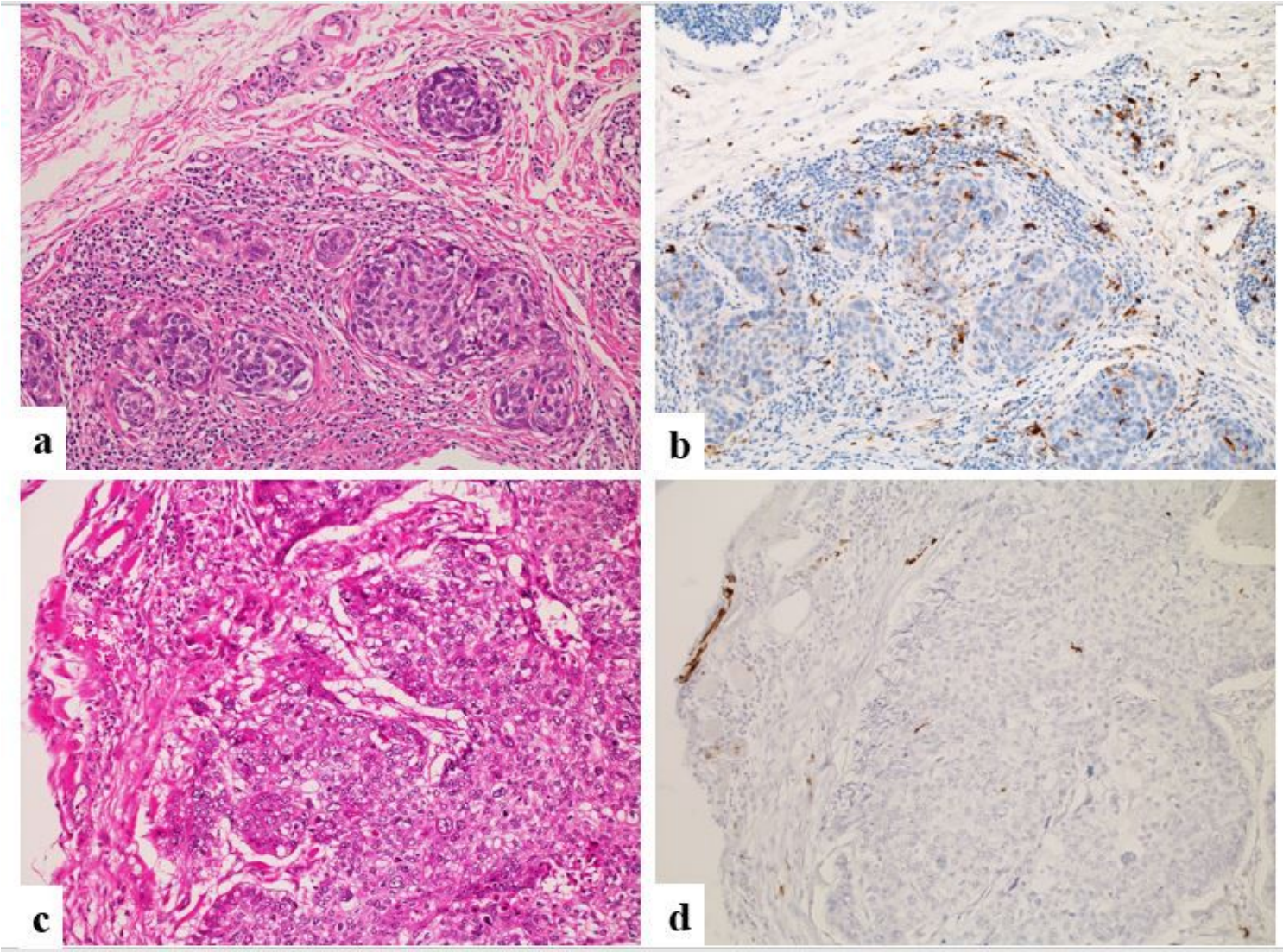

Figure 3

Representative histological images of S100+ DC infiltration in laryngeal squamous cell carcinoma. a: An S100-high case (HE; ×200). b: IHC of S100 in an S100-high case (×200). c: An S100-low case (HE; ×200). d: IHC of S100 in an S100-low case (×200).

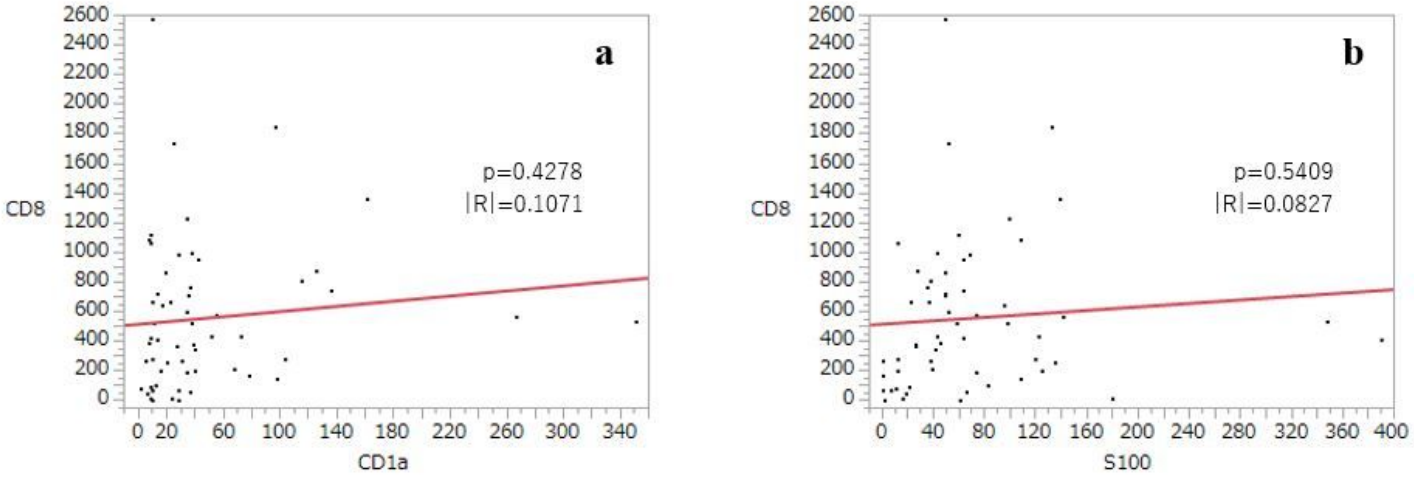

Figure 4

Linear regression analyses (a) between CD1a+ DCs and CD8+ CTLs and (b) between S100+ DCs and CD8+ CTLs.

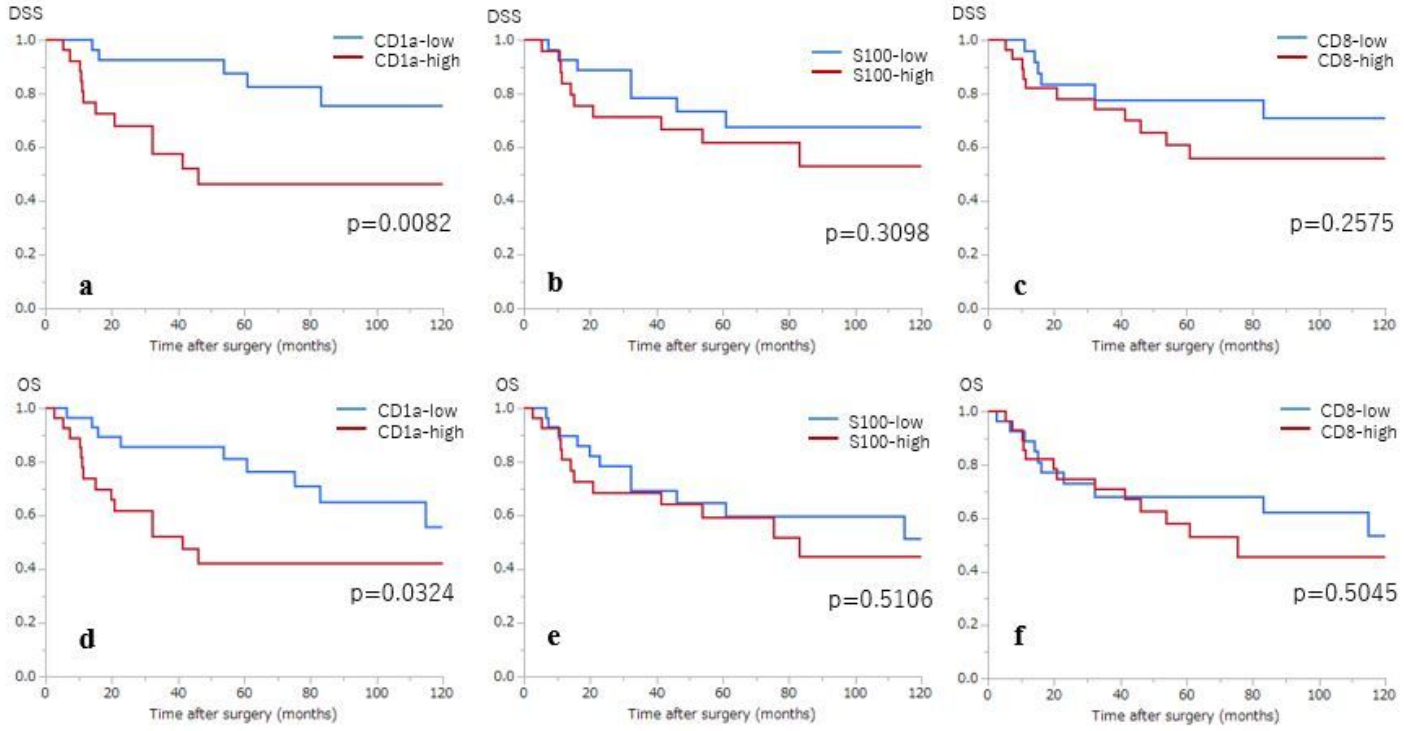

Figure 5

Kaplan-Meier survival curves according to CD1a, S100, and CD8 status in advanced laryngeal cancer. a–c: Disease-specific survival (DSS) according to CD1a, S100, and CD8, respectively. d–f: Overall survival

(OS) according to CD1a, S100, and CD8, respectively.