Impact of lymph node ratio and number of lymph node metastases on survival and recurrence in laryngeal squamous cell carcinoma

Gabriele Molteni MD, PhD, FEBORL-HNS[1,2] | **Riccardo Nocini MD**[2] | **Francesco Mattioli MD**[3] | **Meijin Nakayama MD**[4] | **Rogerio A. Dedivitis MD**[5] | **Giuditta Mannelli MD, PhD**[6] | **Erika Crosetti MD**[7] | **Carlos Miguel Chiesa-Estomba MD**[8] | **Jon A. Sistiaga-Suarez MD**[9] | **Leone Giordano MD**[10] | **Andrea Galli MD**[10] | **Marco Lionello MD**[11] | **Giuseppe Mercante MD**[12,13] | **Massimo Ralli MD**[14] | **Matteo Fermi MD**[15,16] | **Alejandro Klein-Rodríguez MD**[17] | **Miguel Mayo-Yáñez MD**[17] | **Edoardo Serafini MD**[3] | **Francesca Pirola MD**[12,13] | **Alberto Paderno MD**[18] | **Francesca Cambria MD**[14] | **Nobuhiko Oridate MD**[19] | **Daisuke Sano MD**[19] | **Paolo Boscolo-Rizzo MD**[20] | **Alberto Vito Marcuzzo MD**[20] | **Giampiero Parrinello MD**[21] | **Filippo Marchi MD**[21,22] | **Alberto Maria Saibene MD**[23] | **Manuel Tucciarone MD**[24] | **Guillem Viscasillas MD**[25] | **Karol Zeleník MD**[26] | **Daniel Marin Ramos MD**[27] | **Jose Palacios-Garcia MD, MSc, FEBEORL-HNS**[28] | **Giancarlo Tirelli MD**[20] | **Raul Pellini MD**[29] | **Gaetano Paludetti MD**[30,31] | **Jacopo Galli MD**[30,31] | **Giorgia Rossi MD**[30,31] | **Marco De Vincentiis MD**[14] | **Claudio Roberto Cernea MD**[32] | **Giuseppe Spriano MD**[12,13] | **Mario Bussi MD**[10] | **Giorgio Peretti MD**[21,22] | **Giovanni Succo MD**[33] | **Cesare Piazza MD**[18] | **Andy Bertolin MD**[11] | **Luiz Paulo Kowalski MD**[32] | **Livio Presutti MD**[15,16] | **Andrea Sacchetto MD**[2]

Abstract

Introduction: The aim of this study is to assess the impact of lymph node ratio (LNR) and number of positive lymph nodes (NPLN) on mortality and recurrence rates in patients with laryngeal squamous cell carcinoma.

Materials and methods: We conducted a retrospective multicenter international study involving 24 Otorhinolaryngology-Head and Neck Surgery divisions. Disease-specific survival (DSS) and disease-free survival (DFS) were evaluated as the main outcomes. The curves for DSS and DFS according to

NPLN and LNR were analyzed to identify significant variations and establish specific cut-off values.

Results: 2507 patients met the inclusion criteria. DSS and DFS were significantly different in the groups of patients stratified according to LNR and NPLN. The 5-year DSS and DFS based on LNR and NPLN demonstrated an improved ability to stratify patients when compared to pN staging.

Conclusion: Our data demonstrate the potential prognostic value of NPLN and LNR in laryngeal squamous cell carcinoma.

KEYWORDS

laryngeal cancer, lymph node, lymph node metastasis, lymph node ratio, oncology

1 | INTRODUCTION

Squamous cell carcinomas account for 95% of malignant laryngeal tumors. These neoplasms show a marked tendency to spread locally with early involvement of the adjacent lymph node stations.[1] The presence of lymph node metastases is a highly significant negative prognostic factor in patients with laryngeal carcinoma. Several studies have shown that locoregional involvement reduces overall survival (OS) by 30%–50%, albeit with different treatment modalities.[2–4] In addition, lymph node involvement in laryngeal carcinoma significantly increases both the locoregional recurrence rate and the risk of developing distant metastases.[5]

The laryngeal subsites (supraglottis, glottis, and subglottis) show marked differences in terms of the risk of lymph node metastasis.[6,7] Malignant tumors of supraglottic origin have a greater tendency for locoregional involvement; the percentage of lymph node metastases identified in exclusively glottic or subglottic carcinomas is less pronounced.[8,9] The subsite of origin of the tumor is one of the factors that determine the behavior of laryngeal neoplasia. There are certainly other factors, known and unknown, that influence its behavior and local and distant extension. The main risk factors reported in the literature are the degree of tumor differentiation, size of the tumor, size and number of lymph nodes involved in metastases, extracapsular lymph node involvement, presence of intratumoral inflammatory reaction, depth of invasion, and vascular and perineural invasion.[5,10–12]

Lymphatic spread of a laryngeal tumor to regional lymph nodes usually occurs predictably and sequentially at levels II–IV. Several studies[13,14] have defined the preferential patterns of lymph node metastasis. Laryngeal squamous cell carcinomas tend to affect levels I and V at very low percentages, and in almost all cases, there is simultaneous involvement of levels II–IV.[9] Tumors arising in the median supraglottic region and advanced-stage tumors of the glottic region (T3-T4) preferentially metastasize to levels II–III and IV, frequently bilaterally. Early-stage glottic lesions (T1) rarely metastasize to regional lymph nodes.[9] Lateral supraglottic lesions and T2 glottic tumors preferentially metastasize to levels II–III and IV on the same side. In contrast, subglottic tumor extension correlates with the risk of metastasis to level VI pre- and paratracheal lymph nodes. The connection between the lymphatic networks of the hemilarynx at both the supraglottic and subglottic levels has been demonstrated as an anatomical prerequisite for possible metastasis to the contralateral cervical lymph nodes. In contrast, unpredictable lymph node metastases (skip metastases) are quite rare, particularly when compared to other sites in the head and neck region, such as the tongue and oral mucosa.

Recently, attention has been paid to the concept of lymph node ratio (LNR) or lymph node density (LND), which is the ratio of the number of metastatic lymph nodes to the total number of lymph nodes removed during surgical treatment. Recent studies have recognized its importance as a prognostic factor for numerous neoplasms such as carcinomas of the bladder,[15] stomach, and the colorectal and oral cavities.[16,17] Recently, some authors have attempted to determine the prognostic value of LNR in predicting cancer-related mortality of patients with laryngeal carcinoma and positive cervical lymph nodes (pN+).[18–20] All of the studies confirmed the importance of LNR as an independent prognostic predictor of OS and disease-free survival (DFS) in patients with squamous cell carcinoma of the larynx. Other authors have focused their research on the relationship between the absolute number of metastatic lymph nodes and the survival of patients with squamous cell carcinomas[21] and demonstrated that the number of metastatic lymph nodes is an independent factor associated with mortality. Although the 8th edition of the American Joint Committee on Cancer (AJCC) staging system[22] introduced critical

changes to improve stratification of the survival of patients, including incorporating extranodal extension (ENE+) as an upstaging criterion, further modifications have yet to be made regarding the N2 subgroups. Therefore, the cumulative adverse effect of the total metastatic burden may be underestimated, so these modifications may improve prediction ability beyond that currently recognized by the AJCC.

This study aimed to assess the impact of LNR and absolute number of metastatic lymph nodes on mortality and recurrence rates in patients with laryngeal squamous cell carcinoma.

TABLE 1 List of participant hospitals

	Participant hospitals	
1	Azienda Ospedaliera Universitaria Integrata di Verona	Verona, Italy
2	Azienda Ospedaliera Universitaria di Modena	Modena, Italy
3	Policlinico S. Orsola-Malpighi	Bologna, Italy
4	Ospedale di Vittorio Veneto	Vittorio Veneto, Italy
5	IRCCS Candiolo	Torino, Italy
6	Humanitas Research Hospital Milano	Milano, Italy
7	IRCCS San Raffaele Milano	Milano, Italy
8	Spedali Civili di Brescia	Brescia, Italy
9	Azienda Ospedaliera Universitaria Policlinico Umberto I	Roma, Italy
10	Policlinico Universitario Agostino Gemelli IRCCS	Roma, Italy
11	Istituto Nazionale Tumori Regina Elena	Roma, Italy
12	Azienda Ospedaliero Universitaria Careggi	Firenze, Italy
13	Azienda sanitaria universitaria Giuliano Isontina	Trieste, Italy
14	Ospedale di Treviso	Treviso, Italy
15	Ospedale Policlinico Universitario San Martino	Genova, Italy
16	ASST Santi Paolo e Carlo	Milano, Italy
17	Yokohama City University Medical Center	Yokohama, Japan
18	Hospital Universitario MútuaTerrassa	Barcelona, Spain
19	Hospital Universitario de Donostia	Donostia-San Sebastian, Spain
20	Hospital Universitario de Jerez de la Frontera	Jerez de la Frontera, Spain
21	University Hospital Ostrava	Ostrava, Czech Republic
22	Hospital Universitario Virgen Macarena	Sevilla, Spain
23	University Hospital of São Paulo	São Paulo, Brazil
24	Complexo Hospitalario Universitario A Coruña	A Coruña, Spain

2 | MATERIALS AND METHODS

This retrospective multicenter international study involved 24 Otorhinolaryngology-Head and Neck Surgery (OHNS) divisions. The study protocol was approved by the local ethics committee. The recruited centers are listed in Table 1, and the inclusion and exclusion criteria are summarized in Tables 2 and 3, respectively. The following data were collected from the medical records and codified: demographic data, risk factors (alcohol consumption and smoking), treatment modalities (surgery with or without adjuvant treatment), surgical procedures (type of laryngectomy and neck dissection), oncological staging, number of lymph nodes removed, number of metastatic lymph nodes, and follow-up status. Smoking has been reported in packs/year. Selective (SND) or modified radical neck dissections (MRNDs) were performed in adherence with National Comprehensive Cancer Network (NCCN) guidelines. Only patients who underwent at least unilateral neck dissection of three cervical levels were included.

TABLE 2 Inclusion criteria

Inclusion criteria
Patients affected by laryngeal squamous cell carcinoma with any subsite and undergoing surgical treatment
Patients undergoing at least unilateral neck dissection of three cervical levels
Reports available for histology and number of lymph nodes removed
Patients who underwent surgery between 1st January 2005 and 31st December 2019
Patients older than 18

TABLE 3 Exclusion criteria

Exclusion criteria
Patients younger than 18
Patients affected by non-squamous cell carcinoma or other laryngeal malignancies
Patients who underwent radiotherapy, chemoradiotherapy, or neck dissection prior to laryngeal cancer treatment

The AJCC Cancer Staging System, 8th edition was used to define the oncological stage.[22] Preoperative work-up was standardized for all patients and consisted of endoscopic and imaging evaluation. All patients underwent surgery after multidisciplinary team (MDT) discussion and preoperative counseling between head and neck surgeons, and radiation and medical oncologists.

The absolute number of metastatic lymph nodes and the LNR in histological specimens were calculated. Disease-specific survival (DSS) and disease-free survival (DFS) were evaluated as the main outcomes. For DSS, an event was defined as death from recurrence or dissemination of laryngeal cancer. For DFS, the event was any tumor relapse occurring locoregionally or distantly. The curves for DSS and DFS according to the number of metastatic lymph nodes and LNR were analyzed to identify significant variations and establish specific cut-off values.

2.1 | Statistical analysis

The data obtained were analyzed using STATA commercial software (TStat S.r.l., Sulmona, Italy). The median follow-up was calculated using the reverse Kaplan–Meier method. Survival curves were calculated using the Kaplan–Meier method and compared using the log-rank test. Univariate and multivariate analysis was performed using Cox proportional hazards model. The proportional hazard assumption was assessed using scaled Schoenfeld residuals. Stratified Cox regression models were used to account for covariates that violated the proportional hazard assumption. ROC curves were created based on NPLN and LNR values to identify specific cut-offs of DSS and DFS. The AUC (area under the curve) value is a measure of discriminative power of the test, while the closest-to-(0.1) criterion and the Youden index were used to identify the optimal threshold value or cut-off point for the survival. The closest-to-(0,1) criterion defines the optimal threshold as the point minimize the Euclidean distance between the ROC curve and the (0,1) point. The Youden index provides the best trade-off between sensitivity and specificity since maximize the distance of the ROC curve from the diagonal line.

3 | RESULTS

Overall, 2507 patients (mean age 67.5 years, range: 23–94 years), of whom 2224 (89%) were men, met the inclusion criteria. The mean follow-up duration was 48 months (range: 12–191 months). Table 4 summarizes the main features of the recruited patients. The average number of lymph nodes removed was 41 (range: 10–190), and the mean number of metastatic lymph nodes was 1.4 (range: 0–61). The mean LNR was 0.04. A total of 365 patients (14.6%) showed ENE+ of metastatic lymph nodes. The mean number of patients enrolled by the centers is 104, ranging between 26 and 230. The average number of metastatic lymph nodes and LNR were compared among the different centers and no statistically significant difference was found.

DSS and DFS curves were plotted for every feature listed in Table 4. At univariate analysis, the presence of ENE+ correlated with a significant reduction in DSS (hazard ratio [HR] for cancer-related death, 3.7; 95% CI, 3.05–4.7; $p < 0.001$) and DFS (HR for recurrence or death 3.3; 95% CI, 2.77–3.99; $p < 0.001$). Male sex, smoking habit (current or previous smoker), and alcohol consumption (current or previous) were associated with a significant negative effect on DSS ($p < 0.05$), whereas DFS was negatively influenced by male sex ($p < 0.001$) and alcohol consumption ($p < 0.05$).

Glottic origin of the tumor was associated with better survival outcomes (DFS and DSS) than other laryngeal sites ($p < 0.05$). The comparison of treatment protocols revealed statistically significant differences (Table 4). Surgery followed by adjuvant treatment correlated with significantly lower DSS and DFS ($p < 0.001$). Patients with pT4a tumors had poorer DFS and DSS than those with other pT stages ($p < 0.001$). Analysis of survival curves based on pN stage revealed that pN0 patients had better DSS and DFS survival. However, the comparison of survival in the different groups showed that only patients with pN0 and pN3b stages showed significant differences in DSS and DFS when compared with the other groups (Figure 1; $p < 0.05$). No significant differences were observed among patients with different pN2 stages (pN2a, pN2b, or pN2c), as shown in Table 5. Finally, the comparison of patients based on TNM stage showed that stage IV (IVA and IVB) laryngeal cancer was associated with poorer DSS and DFS when compared with other stages ($p < 0.001$). Moreover, stage III laryngeal cancer was associated with a higher risk of recurrence ($p < 0.05$). No other statistically significant differences were found between the other TNM stages.

Multivariate analysis confirmed the significant impact of sex and pN stage on both DSS and DFS (see Table 4). In contrast to the results of the univariate analysis, surgery followed by CRT demonstrated better DSS ($p = 0.003$) and DFS (0.028) when compared with surgery alone. The alcohol consumption and the transglottic origin of the tumor showed poorer DSS, while alcohol consumers and patients affected by pT4a cancer showed worse DFS. Finally, LNR showed significance for both

TABLE 4 Main features of the recruited patients and statistical comparison of DSS and DFS

		Univariate analysis		Multivariate analysis	
Variable	***N* (%)**	**Disease-specific survival**	**Disease-free survival**	**Disease-specific survival**	**Disease-free survival**
Sex					
Male	2224 (88.7%)	Base	Base	Base	Base
Female	283 (11.3%)	HR 0.39; 95% CI, 0.24–0.61; **$p < 0.001$**	HR 0.59; 95% CI, 0.44–0.80; **$p = 0.001$**	HR 0.39; 95% CI, 0.24–0.61; **$p < 0.001$**	HR 0.59; 95% CI, 0.43–0.80; **$p = 0.001$**
Smoking habit					
Non-smoker	237 (9.5%)	Base	Base	Base	Base
Active	1366 (54.5%)	HR 1.55; 95% CI, 1.06–2.3; **$p = 0.024$**	$p = 0.345$	$p = 0.72$	$p = 0.303$
Previous	800 (31.9%)	HR 1.56; 95% CI, 1.03–2.3; **$p = 0.033$**	$p = 0.272$	$p = 0.86$	$p = 0.399$
Missing data	104 (4.1%)				
Alcohol consumption					
None	1398 (55.8%)	Base	Base	Base	Base
Active	705 (28.1%)	HR 1.77; 95% CI, 1.42–2.22; **$p < 0.001$**	HR 1.27; 95% CI, 1.05–1.52; **$p = 0.011$**	HR 1.47; 95% CI, 1.17–1.86; **$p = 0.001$**	$p = 0.542$
Previous	173 (6.9%)	HR 2.64; 95% CI, 1.88–3.69; **$p < 0.001$**	HR 1.79; 95% CI, 1.33–2.39; **$p < 0.001$**	HR 1.93; 95% CI, 1.32–2.81; **$p = 0.001$**	HR 1.52; 95% CI, 1.12–2.05; **$p = 0.006$**
Missing data	231 (9.2%)				
Tumor site					
Supraglottic	975 (38.9%)	Base	Base	Base	Base
Glottic	1107 (44.1%)	HR 0.77; 95% CI, 0.61–0.96; **$p = 0.024$**	HR 0.79; 95% CI, 0.66–0.94; **$p = 0.011$**	$p = 0.127$	$p = 0.382$
Subglottic	45 (1.8%)	$p = 0.837$	$p = 0.326$	$p = 0.870$	$p = 0.351$
Glottic-subglottic	60 (2.4%)	$p = 0.808$	$p = 0.892$	$p = 0.428$	$p = 0.751$
Glottic-supraglottic	175 (7%)	$p = 0.497$	$p = 0.953$	$p = 0.067$	$p = 0.183$
Transglottic	145 (5.8%)	$p = 0.063$	$p = 0.396$	HR 1.66; 95% CI, 1.08–2.55; **$p = 0.021$**	$p = 0.301$
Treatment protocol					
Surgery alone	1256 (50.1%)	Base	Base	Base	Base

TABLE 4 (Continued)

Variable	*N* (%)	Univariate analysis		Multivariate analysis	
		Disease-specific survival	**Disease-free survival**	**Disease-specific survival**	**Disease-free survival**
Surgery + RT	793 (31.6%)	HR 1.81; 95% CI, 1.42–2.30; ***p* < 0.001**	HR 1.56; 95% CI, 1.28–1.88; ***p* < 0.001**	$p = 0.777$	$p = 0.794$
Surgery + RCT	437 (17.5%)	HR 2.63; 95% CI, 2.04–3.40; ***p* < 0.001**	HR 2.19; 95% CI, 1.78–2.70; ***p* < 0.001**	HR 0.60; 95% CI, 0.43–0.83; ***p* = 0.003**	HR 0.74; 95% CI, 0.57–0.97; ***p* = 0.028**
Surgery + CT	21 (0.8%)	HR 4.94; 95% CI, 2.59–9.41; ***p* < 0.001**	HR 3.21; 95% CI, 1.75–5.89; ***p* < 0.001**	$p = 0.145$	$p = 0.067$
Type of surgery					
TLS	252 (10.1%)				
Cordectomy	181				
Supraglottic laryngectomy	71				
OPL	856 (34.2%)				
OPHL type I	229				
OPHL type IIA	355				
OPHL type IIB	181				
OPHL type IIIA	64				
OPHL type IIIB	6				
Other OPL	15				
Vertical partial laryngectomy	6				
Total laryngectomy	1389 (55.4%)				
TORS	4 (0.1%)				
TOUSS	6 (0.2%)				
Neck dissection					
Unilateral	637 (25.4%)				
Bilateral	1870 (74.6%)				
Type of neck dissection					
SND	3837 (87.7%)				
MRND	428 (9.8%)				
RND	112 (2.5%)				

(Continues)

TABLE 4 (Continued)

Variable	*N* (%)	Univariate analysis		Multivariate analysis	
		Disease-specific survival	**Disease-free survival**	**Disease-specific survival**	**Disease-free survival**
T stage					
pT1	149 (5.9%)	Base	Base	Base	Base
pT2	440 (17.6%)	$p = 0.9$	$p = 0.9$	$p = 0.456$	$p = 0.742$
pT3	1033 (41.2%)	$p = 0.113$	HR 1.61; 95% CI, 1.03–2.52; **$p = 0.035$**	$p = 0.782$	$p = 0.434$
pT4A	878 (35%)	HR 3.16; 95% CI, 1.80–5.55; **$p < 0.001$**	HR 2.83; 95% CI, 1.81–4.42; **$p < 0.001$**	$p = 0.151$	HR 1.98; 95% CI, 1.08–3.62; **$p = 0.027$**
pT4B	7 (0.3%)				
N stage					
pN0	1362 (54.3%)	Base	Base	Base	Base
pN1	299 (11.9%)	HR 2.92; 95% CI, 2.16–3.96; **$p < 0.001$**	HR 2.20; 95% CI, 1.72–2.81; **$p < 0.001$**	HR 3.14; 95% CI, 2.27–4.36; **$p < 0.001$**	HR 2.21; 95% CI, 1.70–2.87; **$p < 0.001$**
pN2a	201 (8%)	HR 3.75; 95% CI, 2.29–6.16; **$p < 0.001$**	HR 2.91; 95% CI, 1.92–4.40; **$p < 0.001$**	HR 3.46; 95% CI, 2.04–5.86; **$p < 0.001$**	HR 2.77; 95% CI, 1.81–4.25; **$p < 0.001$**
pN2b	241 (9.6%)	HR 3.60; 95% CI, 2.63–4.93; **$p < 0.001$**	HR 2.64; 95% CI, 2.04–3.43; **$p < 0.001$**	HR 3.11; 95% CI, 2.14–4.50; **$p < 0.001$**	HR 2.20; 95% CI, 1.63–2.98; **$p < 0.001$**
pN2c	163 (6.5%)	HR 4.56; 95% CI, 3.22–6.46; **$p < 0.001$**	HR 3.13; 95% CI, 2.33–4.21; **$p < 0.001$**	HR 3.41; 95% CI, 2.19–5.31; **$p < 0.001$**	HR 2.33; 95% CI, 1.60–3.40; **$p < 0.001$**
pN3a	9 (0.4%)	$p = 0.234$	$p = 0.065$	$p = 0.134$	$p = 0.056$
pN3b	232 (9.3%)	HR 6.27; 95% CI, 4.71–8.35; **$p < 0.001$**	HR 5.01; 95% CI, 3.98–6.30; **$p < 0.001$**	HR 5.59; 95% CI, 3.96–7.88; **$p < 0.001$**	HR 4.15; 95% CI, 3.13–5.50; **$p < 0.001$**
ENE					
ENE−	2141 (85.4%)	Base	Base	Base	Base
ENE+	365 (14.6%)	HR 3.78; 95% CI, 3.04–4.70; **$p < 0.001$**	HR 3.32; 95% CI, 2.77–3.99; **$p < 0.001$**	$p = 0.919$	$p = 0.575$
M stage					
M0	2500 (99.7%)	Base	Base		
M1	7 (0.3%)	$p = 0.778$	$p = 0.086$		
TNM stage					
I	126 (5%)	Base	Base	Base	Base
II	290 (11.6%)	$p = 0.357$	$p = 0.357$	$p = 0.337$	$p = 0.268$

TABLE 4 (Continued)

Variable	*N* (%)	Univariate analysis		Multivariate analysis	
		Disease-specific survival	**Disease-free survival**	**Disease-specific survival**	**Disease-free survival**
III	810 (32.3%)	$p = 0.057$	HR 2.35; 95% CI, 1.15–4.79; **$p = 0.019$**	$p = 0.351$	$p = 0.214$
IVA	1016 (40.5%)	HR 6.23; 95% CI, 2.31–16.77; **$p < 0.001$**	HR 4.39; 95% CI, 2.17–8.88; **$p < 0.001$**	$p = 0.193$	$p = 0.197$
IVB	258 (10.3%)	HR 14.66; 95% CI, 5.37–40.01; **$p < 0.001$**	HR 10.54; 95% CI, 5.14–21.61; **$p < 0.001$**	$p = 0.113$	$p = 0.107$
IVC	7 (0.3%)				
Follow-up status					
Alive without disease	1498 (59.7%)				
Alive with disease	203 (8.1%)				
Dead from disease	385 (15.4%)				
Dead from other cause	421 (16.8%)				
Type of recurrence					
Local	143 (24.3%)				
Regional	81 (13.8%)				
Locoregional	114 (19.4%)				
Distant	148 (25.2%)				
Locoregional and distant	102 (17.3%)				

Note: $p < 0.05$ shown in boldface.

Abbreviations: CI, confidence interval; CT, chemotherapy; DFS, disease-free survival; DSS, disease-specific survival; ENE, extranodal extension; HZ, hazard ratio; MRND, modified radical neck dissection; OPHL, open partial horizontal laryngectomy; OPL, open partial laryngectomy; RCT, radiochemotherapy; RND, radical neck dissection; RT, radiotherapy; SND, selective neck dissection; TLS, transoral LASER surgery; TORS, transoral robotic surgery; TOUSS, transoral endoscopic ultrasonic surgery.

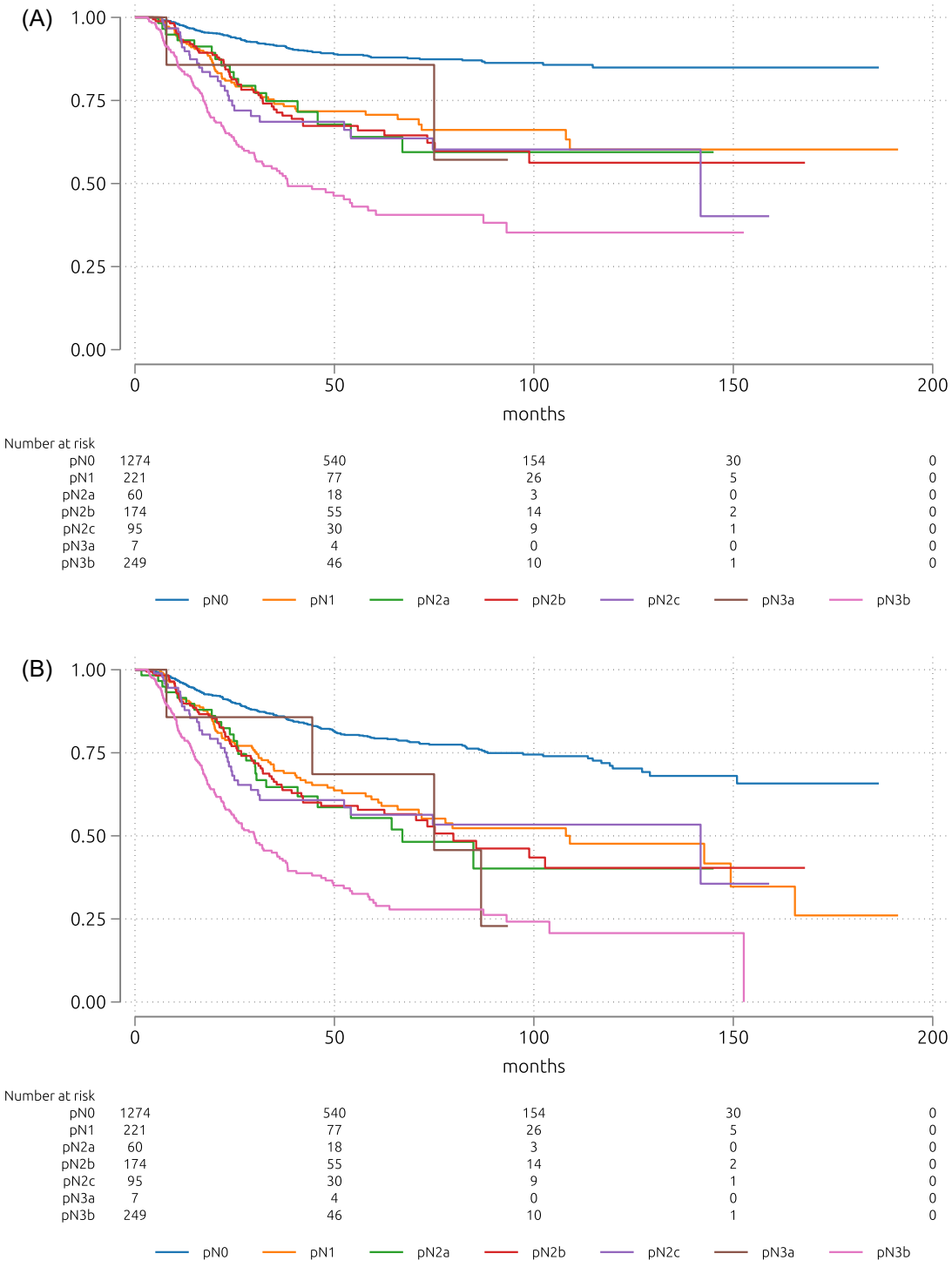

FIGURE 1 Disease-specific survival (A) and disease-free survival curves (B) according to pN stage [Color figure can be viewed at wileyonlinelibrary.com]

DFS and DSS ($p < 0.001$) while NPLN was not a significant variable at multivariate analysis ($p = 0.494$ for DSS and $p = 0.133$ for DFS).

An increased number of metastatic lymph nodes and increased LNR correlated with poorer DSS and DFS; both outcomes continuously increased without a plateau. ROC curves were created in order to identify the optimal value of NPLN and LNR to predict DSS and DFS. This method did not prove accurate as no single value of NPLN and NPLN was found with sensitivity and specificity greater than 70%. However, the relationship between DSS, DFS, LNR, and NPLN was nonlinear, and specific points of variation with a significantly increased risk of disease-specific death and recurrence were identified using Cox

TABLE 5 Five-year DSS and DFS according to pN stage

	DSS (%)	DFS (%)
pN0	81	72
pN1	70	62.5
pN2a	63	55
pN2b	59	54
pN2c	54	47
pN3b	34	30

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival.

proportional hazards models. The cut-offs for LNR were 0.1, 0.2, and 0.4, and the cut-offs for NPLN were 5, 10, and 20. Based on the NPLN cut-offs, specific patient groups were identified: group 0 (57%), group 1–5 (35%), group 6–10 (5%), group 11–20 (2%), group >20 (1%). Similarly, LNR cut-offs were used to identify four groups: group 0 (57%), group 0–0.1 (29%), group 0.1–0.2 (8%), group 0.2–0.4 (4%), and group >0.4 (2%). These cut-off values were subsequently used to obtain different DSS and DFS curves, as shown in Figures 2 and 3, respectively. The 5-year DSS and DFS based on these cut-off values are summarized in Tables 6 and 7. DSS and DFS were significantly different in the groups of patients stratified according to both LNR and NPLN. In particular, patients with LNRs of 0, 0–0.1, 0.1–0.2, and >0.2 showed significant differences in DSS and DFS. Similarly, for NPLN, patients with 0, 1–5, and >5 positive lymph nodes showed statistically significant differences in the survival curves ($p < 0.05$).

Using the same cut-off values, survival curves were generated to differentiate patients based on the site of origin of the malignancy. The number of patients with subglottic tumors was insufficient to obtain significant results. In patients with supraglottic carcinoma, the survival curves (DSS and DFS) and statistical comparisons according to NPLN and LNR were similar to those reported in the total population (Figure 4). When compared with the total population, DFS and DSS in patients with glottic carcinoma showed some significant differences. There were no differences in DFS between patients with 1–5 and 6–10 metastatic lymph nodes ($p = 0.13$), resulting in three different groups: patients with 0, 1–10, and >10 metastatic lymph nodes. A similar result was found when considering DSS analysis. The analysis of DSS and DFS based on LNR in the glottic population revealed three different groups: patients with LNRs of 0, 0–0.4, and >0.4 (Figure 5).

4 | DISCUSSION

Laryngeal squamous cell carcinoma is the only cancer entity worldwide with worsening survival rates over the past several decades.[23] Regional lymph node metastases, advanced-stage disease, surgical resection margins, and extracapsular involvement are established risk factors for locoregional recurrence or distant metastasis in laryngeal and hypopharyngeal carcinomas.[23,24] Studying the role of nodal metastases in head and neck cancer is a key area of research to increase our understanding of tumor behavior and to improve treatment modalities. AJCC nodal staging of the larynx is mainly based on the anatomical location of the lymph nodes (single or multiple, site, and size), without considering the absolute number and ratio of positive lymph nodes.[22] The limitations of the current laryngeal TNM staging system are highlighted by the data reported in our case study, which agrees with those published in similar studies.[18,21,23,25,26] In particular, the comparison of our patients based on pTNM revealed that there were no significant differences in terms of DSS and DFS in patients with stages I–III disease and that the only significant differences were observed for stages IVA and IVB.

Furthermore, when analyzing the pN stage, no differences were observed in DFS and DSS in patients with stages pN1 to pN2c disease. These data are consistent with those obtained by Ho et al.[21] In a cohort of 8351 patients, the reported 3-year OS rates were very similar in pN2 stage patients: 52.8% in patients with pN2a, 55.1% with pN2b, and 48.7% with pN2c. These findings cast doubt on the predictive validity of the current TNM laryngeal staging system. Ho and colleagues[21] also proposed an alternative nodal staging classification considering only the absolute number of lymph node metastases and the presence of ENE, obtaining a more accurate prediction of OS when compared to TNM staging. Similarly, Choi et al. proposed a new classification system for laryngeal and hypopharyngeal tumors treated with surgery, including the number of positive nodes, and this improved OS and DSS prediction compared to both the 7th and 8th Editions of the TNM staging system.[27] Moreover, Marchi et al. analyzed DFS and reported that the impact of the number of lymph node metastases was a detrimental prognostic factor at multivariable analysis with an HR of 1.2 ($p = 0.002$), indicating an increase in risk of 20% for each positive node detected.[5]

ENE is frequently reported as one of the most significant prognostic factors in head and neck cancers.[24] Our data obtained at the univariate analysis also emphasize the great difference in prognostic terms between ENE+ and ENE− patients. The nonsignificance of ENE on multivariate analysis appears to be attributable to omitted variable bias. Since 86% of ENE+ patients had stage pN3b, the influence of the variable ENE is indistinguishable from that of stage pN. The presence of ENE is a strong predictor of both DSS and DFS, and adjuvant treatment is mandatory to improve overall, disease-free, and disease-specific survival.

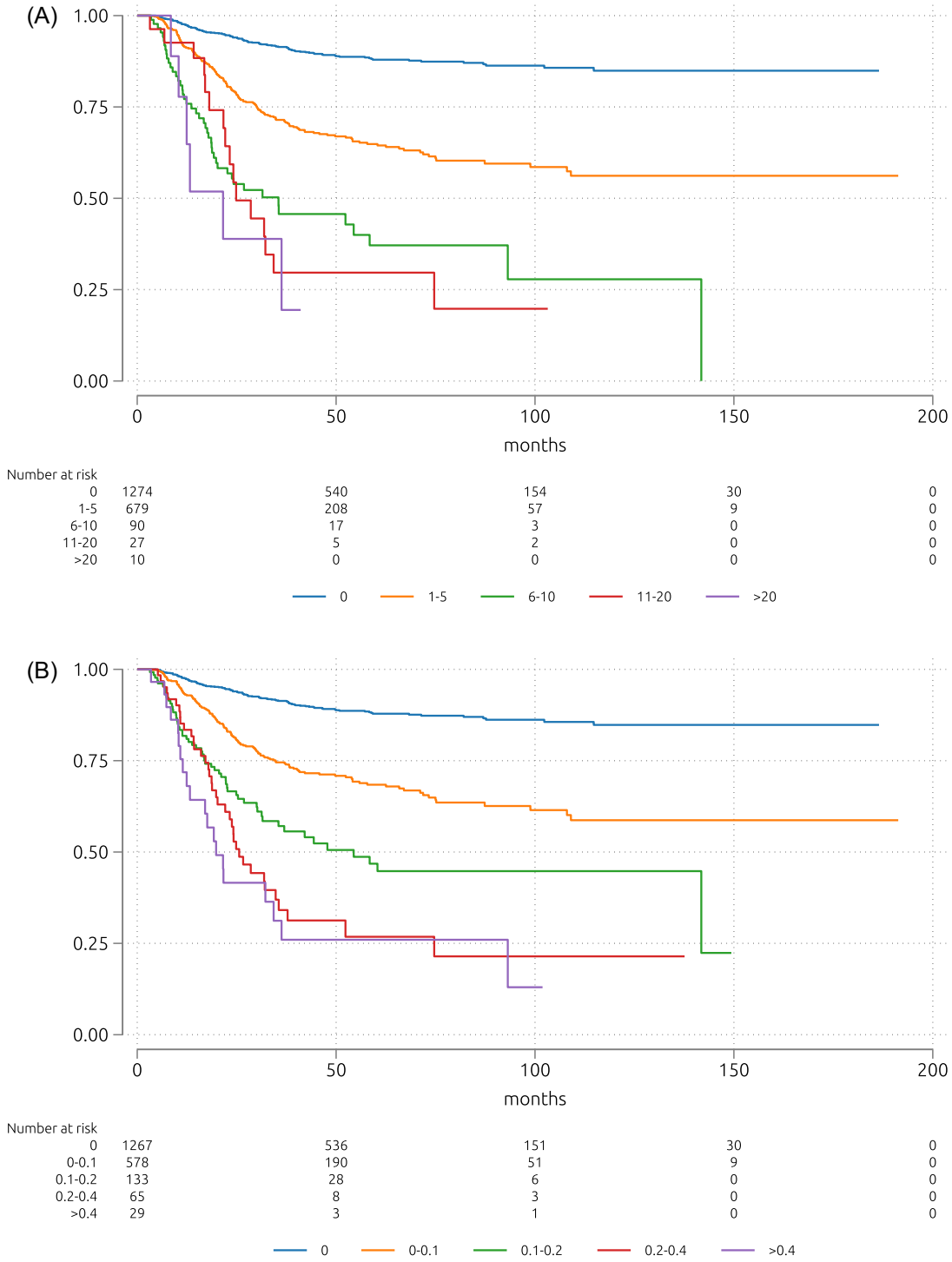

FIGURE 2 Disease-specific survival curves according to number of metastatic lymph nodes (A) and lymph node ratio (B) [Color figure can be viewed at wileyonlinelibrary.com]

Interesting findings on the impact of lymph node metastasis have been reported by Wang et al.[26] who conducted a complex analysis of overall and cancer-specific survival in patients with laryngeal cancer, considering three parameters: LNR, logarithmic ratio of positive lymph nodes (LODDS), and NPLN. The authors also developed improved prognostic models and demonstrated that NPLN combined with LODDS was the optimal prognostic choice. They proposed a predictive nomogram that demonstrated a better predictive performance than the 7th AJCC TNM staging system. This thorough analysis demonstrated the need to integrate

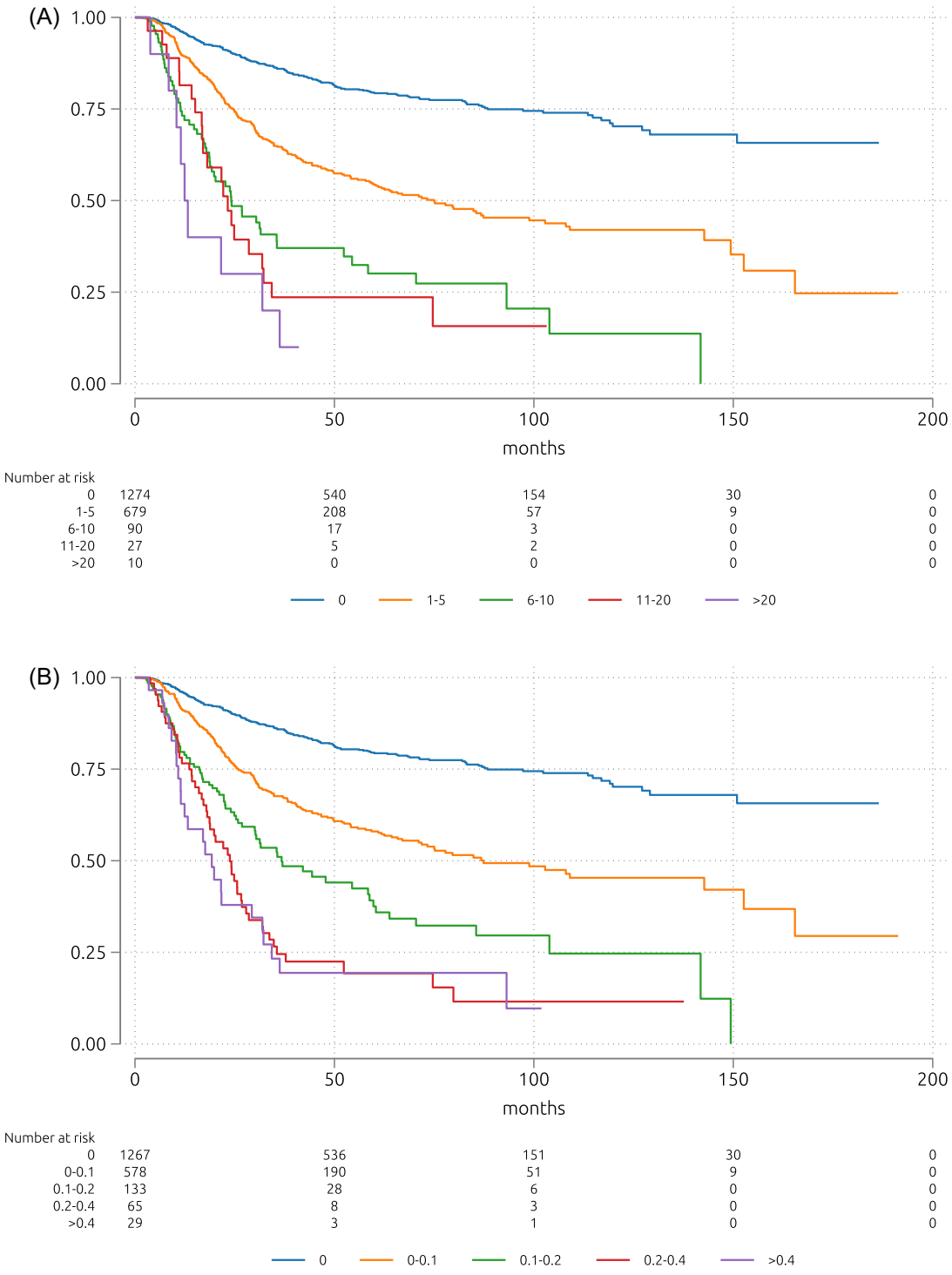

FIGURE 3 Disease-free survival curves according to number of metastatic lymph nodes (A) and lymph node ratio (B) [Color figure can be viewed at wileyonlinelibrary.com]

new prognostic factors with TNM to achieve a better definition of laryngeal carcinoma.

To the best of our knowledge, this study is the first to analyze the impact of the absolute number of lymph nodes and LNR on mortality and DFS in malignant tumors of the larynx using a large sample size. Several earlier studies have attempted to identify specific cut-offs for both LNR and NPLN to better estimate survival in head and neck cancer. A limited number of studies have conducted this analysis exclusively on patients with laryngeal cancer.[18–21,25–28] Currently, there is no consensus about the significant values for LNR and NPLN that could be used in clinical practice for

TABLE 6 Five-year DSS and DFS according to number of metastatic lymph nodes

	DSS (%)	DFS (%)
0	81	72
1–5	72	56
6–10	55	35
11–20	32	10
>20	16	2

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival.

TABLE 7 Five-year DSS and DFS according to LNR

	DSS (%)	DFS (%)
0	81	72
0–0.1	75	62
0.1–0.2	65	42
0.2–0.4	46	24
>0.4	33	13

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival; LNR, lymph node ratio.

oncological staging and prognosis. A study conducted by Imre et al.[20] on 101 patients showed that the LNR cut-off value for a meaningful separation was 0.09. The presence of an LNR > 0.09 was the only significant independent prognostic factor for OS and DFS. Similar data were reported by Künzel et al.[19] who stated that patients with LNR > 0.09 had a hazard ratio of 2.065 for a DSS event compared to LNR < 0.09. Similarly, we identified a cut-off of 0.1 as one of the meaningful values for stratification of DSS and DFS. However, identification of a single significant value was not possible because of the progressive worsening of DSS and DFS with the increase in LNR. For example, Wang et al.[28] defined three different groups based on LNR (<0.09, 0.09–0.2, and >0.2) and showed that stratification of DSS and OS based on these cut-offs is more accurate than N stage according to TNM.

A critical analysis of the role of LNR and the number of nodal metastases conducted by de Ridder et al.[29] confirmed that LNR is a prognostic factor in head and neck cancer. However, the adequacy and extent of neck dissection are surgeon-dependent, while the rate of detected metastases may be influenced by the accuracy of the pathological evaluation.[30] De Ridder et al.[29] stated that NPLN is as accurate as the LNR and less susceptible to variation if lymph node sampling is adequate. Standardization of lymph node dissection and subsequent histological analysis would be desirable to obtain more objective data and limit bias. Moreover, lymphatic tumor spread is supported by tumor-associated lymphogenesis, and the number of lymph nodes excised during neck dissection may be important for understanding the tumor biology characteristics.[31]

In the international literature, there is a lack of studies that systematically address the influence of NPLN on survival in laryngeal cancer. The above-mentioned study conducted by Ho and colleagues[21] presented significant evidence regarding the impact of NPLN on overall mortality but nevertheless had the major limitation of having considered patients with laryngeal or hypopharyngeal neoplasms. These tumors are clearly characterized by significantly different behaviors and prognoses; thus, the results reported by Ho et al.[21] are not comparable to those in our study.

Our study only included patients with laryngeal malignancies undergoing surgical treatment and involved a retrospective evaluation of all the parameters discussed. Consequently, only pathological staging was considered, which may not fully translate to clinical staging. Although only experienced, high-volume centers were involved, the patients enrolled were heterogeneous in terms of both characteristics and treatment modalities. The heterogeneity of the surgical procedures performed also resulted in a significant difference in the number of lymph nodes removed. Based on our inclusion criteria, only patients who underwent at least unilateral neck dissection at the three cervical levels were included. However, the number of cervical lymph nodes also differs between patients with tumors and healthy individuals.[32] To our best knowledge, the minimum and the optimal number of lymph nodes removed during neck dissection in patients with laryngeal carcinoma has not been identified, and only a very limited number of studies have attempted to analyze the influence of this factor on oncological outcomes.[33,34] Improved prognosis following more extensive lymphadenectomy has been demonstrated in colon, breast, gastric, esophageal and bladder cancer, but not in head and neck carcinomas. The standardization of the cervical dissection is essential to allow the adequate evaluation of NRLN and NPLN and to enable the application of these factors in the prognostic paradigm of patients with laryngeal carcinoma.

When surgery is chosen as the treatment modality for laryngeal carcinoma, several factors are considered to define the indication for adjuvant treatment. Currently, in the absence of other adverse factors, adjuvant radiotherapy or radiochemotherapy is only indicated for patients with positive margins or with stage pN2, pN3, or ENE+ neoplasia. Our data obtained from the univariate analysis show that DSS and DFS are significantly worse ($p < 0.01$) in patients receiving adjuvant radiotherapy or radiochemotherapy than in patients undergoing surgery alone. This finding must be analyzed critically and is in the first instance attributable to the worse stage of disease of patients requiring adjuvant treatment. Indeed, multivariate analysis showed that DSS and DFS were

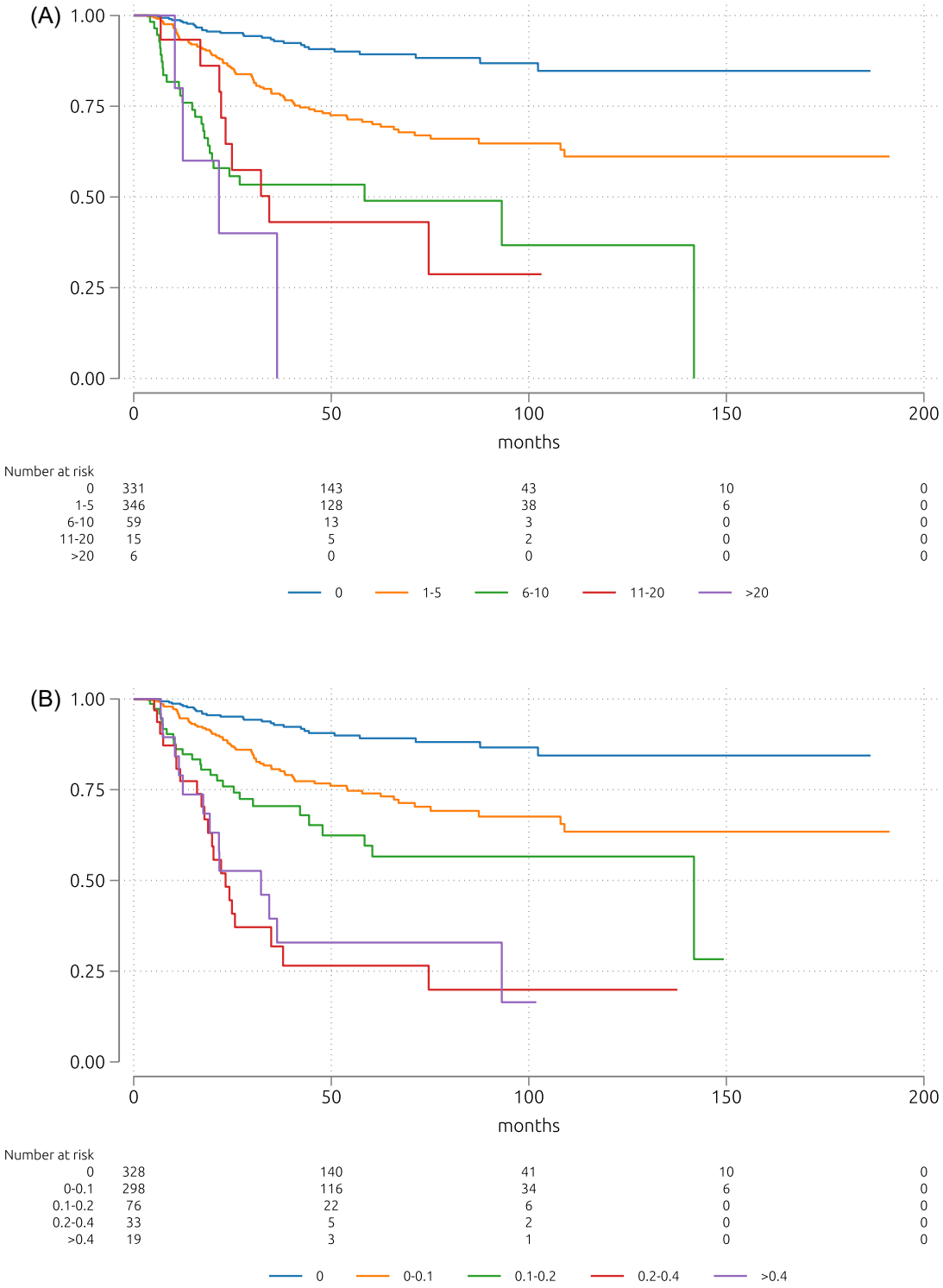

FIGURE 4 Disease-specific survival curves in patients affected by supraglottic (A, B) and glottic (C, D) cancer, according to number of metastatic lymph nodes and lymph node ratio [Color figure can be viewed at wileyonlinelibrary.com]

significantly better ($p = 0.003$ and $p = 0.028$) in patients undergoing surgery and adjuvant radiochemotherapy, underlining the positive impact of these combined treatments. Identifying factors that can guide the choice of a specific treatment protocol for each patient is crucial and the evidence observed in our study and others[21,26] regarding NPLN and LNR supports their inclusion in the treatment decision paradigm. We hope that further study will clarify whether this very specific subset of patients might benefit from adjuvant treatments.

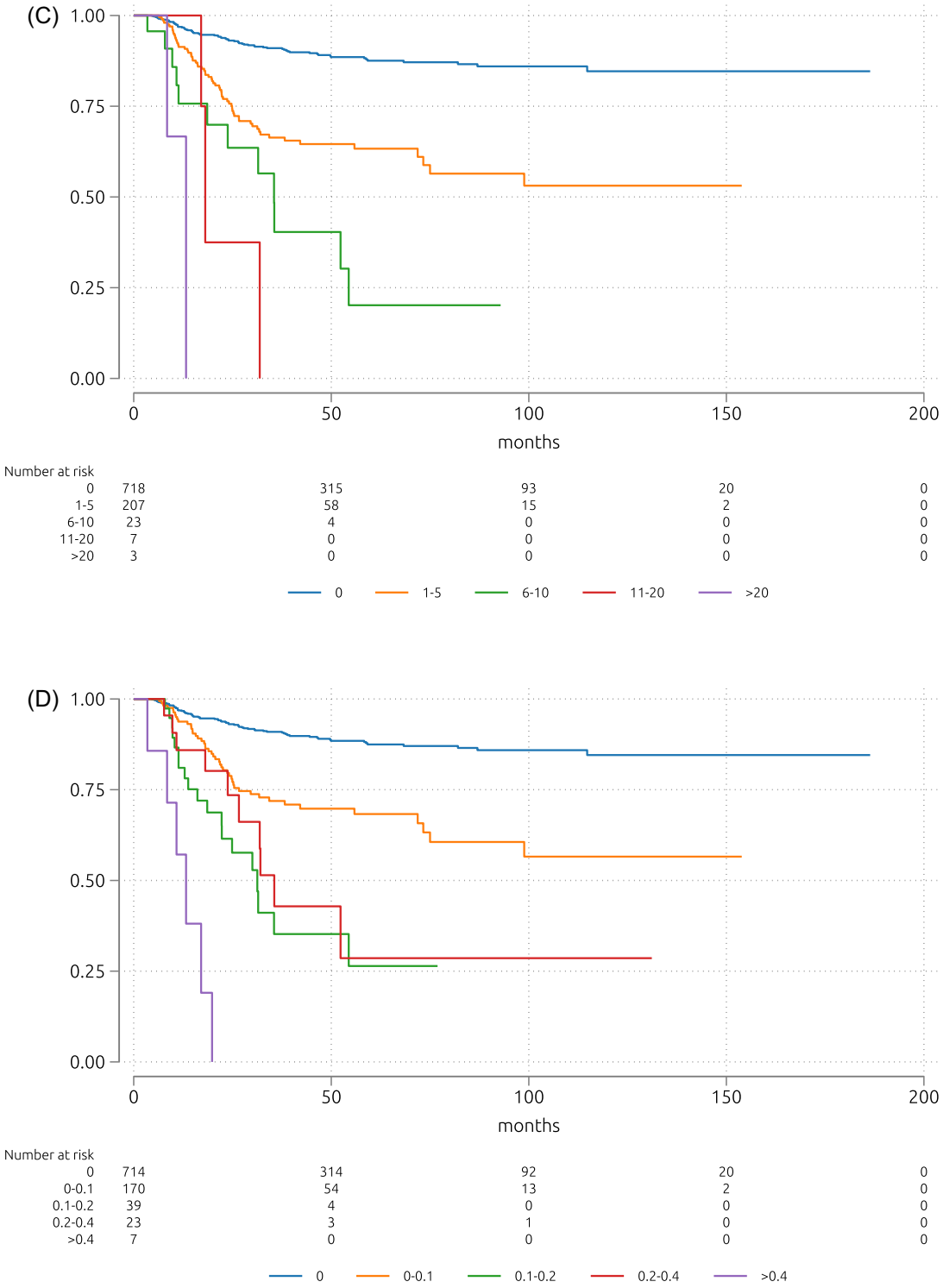

FIGURE 4 (Continued)

The retrospective nature of this study is its main limitation. The retrospective enrolment of patients is burdened by a possible selection bias, as privacy regulations in different cities and countries of the authors may differ. The centers involved followed local regulations as set by individual ethics committees. Although obtaining informed consent is not usually required in a retrospective setting, in some countries it was still required according to local privacy regulations. This may have unintentionally led to the selection of patients with a better prognosis. Similarly, the addition of adjuvant treatments was discussed on a case-by-case basis by the originating MDT and their use cannot be

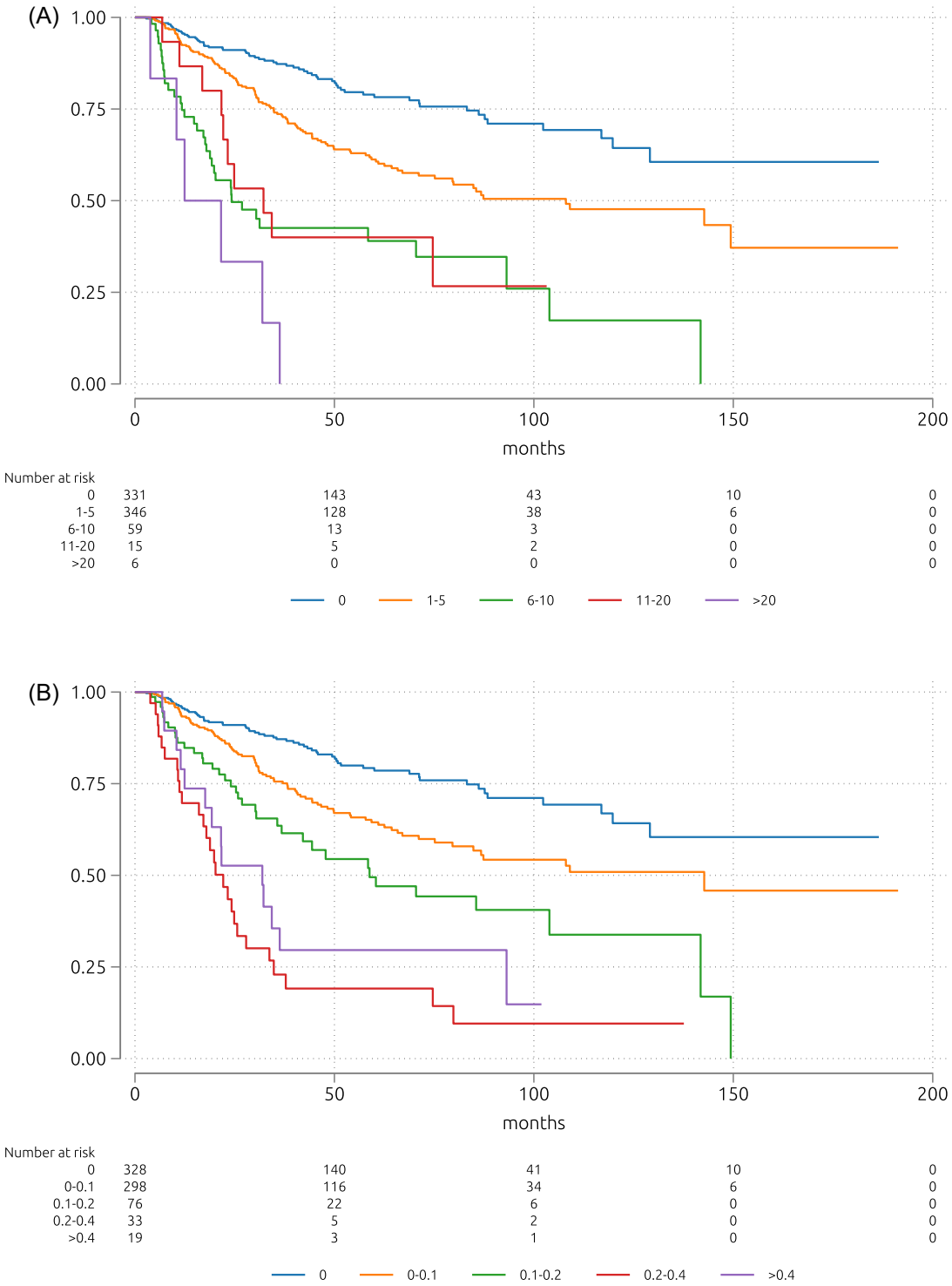

FIGURE 5 Disease-free survival curves in patients affected by supraglottic (A, B) and glottic (C, D) cancer, according to number of metastatic lymph nodes and lymph node ratio [Color figure can be viewed at wileyonlinelibrary.com]

unequivocally established, as this would require a prospective randomized trial. In such a setting, several interesting pathological factors could be studied such as the lymph node size, the micro- versus macro-ENE, and the laryngeal depth of invasion, to advocate the need for adjuvant treatment.

The prediction of tumor behavior or at least the identification of high-risk neoplasms should be the main goals to optimize the current treatment modalities and to hopefully improve the survival rates in patients with laryngeal carcinoma.

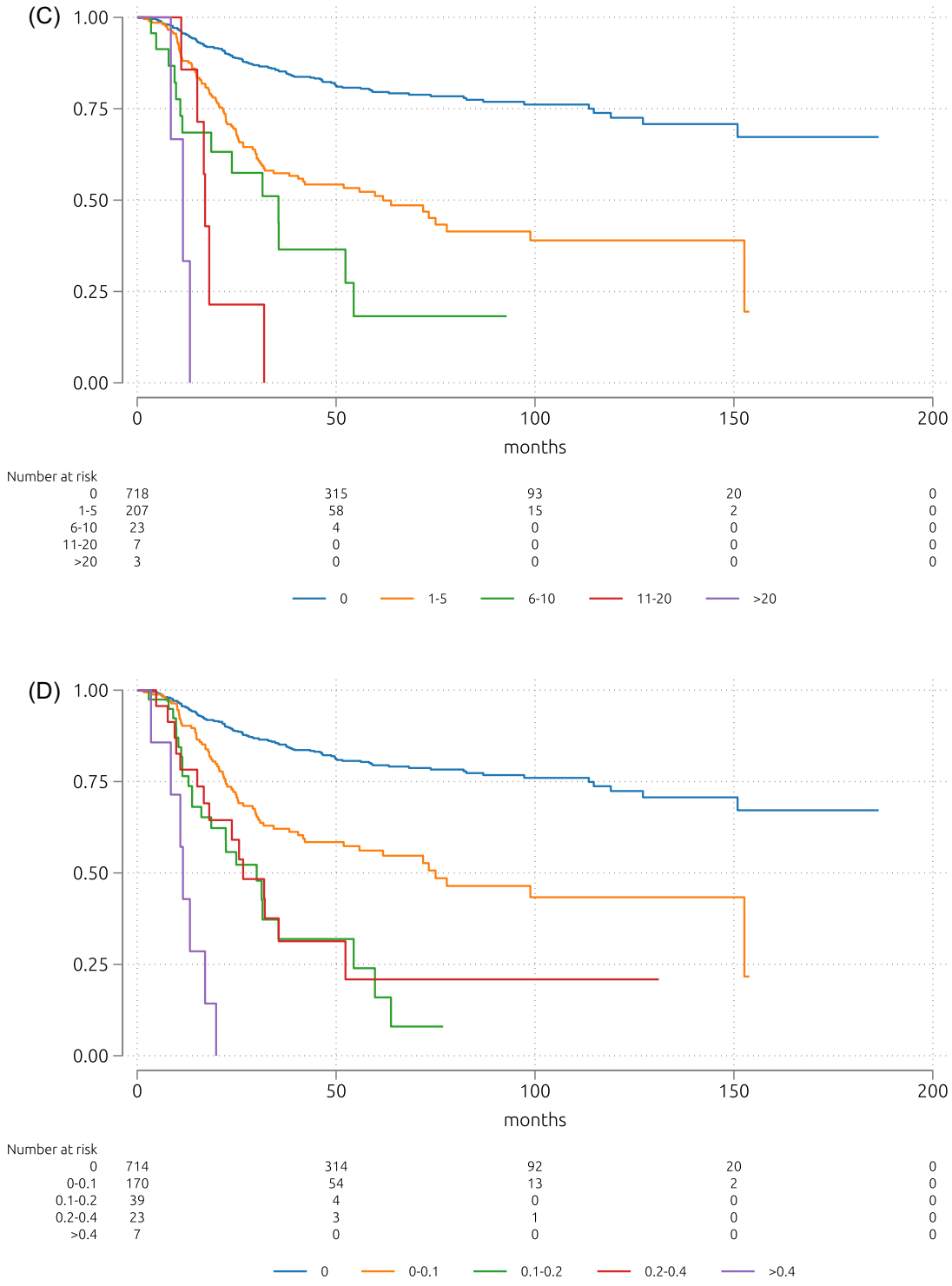

FIGURE 5 (Continued)

5 | CONCLUSION

This study provides strong evidence and important insights into nodal staging. Our data demonstrate the prognostic value of NPLN and LNR in laryngeal squamous cell carcinoma. The inclusion of these factors should be considered together with pTNM staging to improve the risk stratification of patients with laryngeal cancer and provide important information about survival and indications for potential adjuvant

treatment. However, the integration of NPLN and LNR in the decision-making process for adjuvant therapies must be prospectively evaluated in a large series of patients before implementation in clinical practice.

AFFILIATIONS

[1]Department of Otolaryngology – Head and Neck Surgery, University Hospital of Verona, Verona, Italy
[2]Unit of Otolaryngology, Department of Surgery, Dentistry and Pediatrics, University of Verona, Verona, Italy
[3]Department of Otorhinolaryngology – Head and Neck Surgery, University Hospital of Modena, Modena, Italy
[4]Department of Otolaryngology, Yokosuka Kyosai Hospital, Yokohama, Japan
[5]Department of Head and Neck Surgery, University of Sao Paulo School of Medicine, Sao Paulo, Brazil
[6]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
[7]Department of Otorhinolaryngology, San Luigi Gonzaga Hospital, University of Turin, Turin, Italy
[8]Department of Otorhinolaryngology – Head and Neck Surgery, Donostia University Hospital, Biodonostia Research Institute, Faculty of Medicine, Deusto University, San Sebastian, Spain
[9]Department of Otorhinolaryngology – Head and Neck Surgery, Donostia University Hospital, San Sebastian, Spain
[10]Department of Otolaryngology – Head and Neck Surgery, San Raffaele Scientific Institute, Vita-Salute University, Milan, Italy
[11]Unit of Otorhinolaryngology, Vittorio Veneto Hospital, Vittorio Veneto, Italy
[12]Unit of Otorhinolaryngology, IRCCS Humanitas Research Hospital, Milan, Italy
[13]Department of Biomedical Sciences, Humanitas University, Milan, Italy
[14]Department of Sense Organs, Sapienza University of Rome, Rome, Italy
[15]Department of Otorhinolaryngology – Head and Neck Surgery, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[16]Department of Specialist, Diagnostic and Experimental Medicine (DIMES), Alma Mater Studiorum, Università di Bologna, Bologna, Italy
[17]Department of Otorhinolaryngology – Head and Neck Surgery, Complexo Hospitalario Universitario A Coruña, A Coruña, Spain
[18]Unit of Otorhinolaryngology – Head and Neck Surgery, ASST Spedali Civili Brescia, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia, Italy
[19]Department of Otolaryngology – Head and Neck Surgery, Yokohama City University School of Medicine, Yokohama, Japan
[20]Section of Otolaryngology, Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy
[21]Department of Otorhinolaryngology – Head and Neck Surgery, IRCCS Ospedale Policlinico San Martino, Genoa, Italy
[22]Department of Surgical Sciences and Integrated Diagnostics (DISC), University of Genoa, Genoa, Italy
[23]Unit of Otolaryngology, Santi Paolo e Carlo Hospital, Department of Health Sciences, Università degli Studi di Milano, Milan, Italy
[24]Department of Otorhinolaryngology, University Hospital of Jerez, Cádiz, Spain
[25]Department of Otolaryngology – Head and Neck, Althaia, Xarxa Assitencial Universitaria de Manresa, Barcelona, Spain
[26]Department of Otorhinolaryngology – Head and Neck Surgery, University Hospital Ostrava, Ostrava, Czech Republic
[27]Department of Head and Neck Surgery, Cancer Institute of São Paulo ICESP, São Paulo, Brazil
[28]Department of Otorhinolaryngology – Head and Neck Surgery, Hospital Universitario Virgen Macarena, Seville, Spain
[29]Department of Otolaryngology – Head and Neck Surgery, IRCCS Regina Elena National Cancer Institute, Rome, Italy
[30]Unit of Otorhinolaryngology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[31]Department of Head and Neck and Sensory Organs, Università Cattolica del Sacro Cuore, Rome, Italy
[32]Department of Head and Neck Surgery, University of São Paulo Medical School, São Paulo, Brazil
[33]Unit of Otolaryngology – Head and Neck Surgery, San Giovanni Bosco Hospital, Turin, Italy

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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