Medicine®

OBSERVATIONAL STUDY

OPEN

Preoperative SCC Antigen, CRP Serum Levels, and Lymph Node Density in Oral Squamous Cell Carcinoma

Mohamad Adel, MD, MSc, Chung-Kan Tsao, MD, Fu-Chan Wei, MD, Huei-Tzu Chien, PhD, Chih-Hsiung Lai, PhD, Chun-Ta Liao, MD, Hung-Ming Wang, MD, Kang-Hsing Fan, MD, Chung-Jan Kang, MD, Joseph Tung-Chieh Chang, MD, MHA, and Shiang-Fu Huang, MD, PhD

Abstract: The prognostic significance of squamous cell carcinoma antigen (SCC-Ag) and C-reactive protein (CRP) levels and lymph node density (LND) has been individually recognized in oral squamous cell carcinoma (OSCC). We investigated the relationship between preoperative serum markers (SCC-Ag and CRP) and postoperative prognostic marker (LND) in this study. We retrospectively analyzed 277 OSCC patients who underwent primary curative resection and neck dissection with/or without adjuvant therapy between March 2008 and November 2013. Serum SCC-Ag and CRP levels were measured preoperatively. Distant metastasis, overall survival (OS), and disease-free survival (DFS) were used to evaluate the prognostic significance of preoperative SCC-Ag and CRP levels in relation to LND. LND (cutoff point $\geq$0.06) correlated with the pathologic tumor status, pathologic nodal metastasis, degree of differentiation, tumor stage, tumor depth ($\geq$10 mm vs $<$10 mm), and perineural invasion (all P values were $<$0.001). LND was significantly associated with development of distant metastasis, DFS, and OS (all P values were $<$0.001). Preoperative elevated CRP and SCC-Ag levels were significantly associated with LND ($P = 0.006$), DFS ($P < 0.001$), and OS ($P < 0.001$). LND$^+$ patients were further stratified into prognostic groups according to their SCC-Ag and CRP levels (DFS: $P = 0.010$; OS: $P = 0.003$). LND correlated with the incidence of DM, DFS, and OS in patients with OSCC. Concurrent elevated preoperative SCC-Ag and CRP levels are predictors for LND. In addition, SCC-Ag and CRP are markers for classifying high-risk LND$^+$ patients with OSCC into subgroups.

Editor: Li W. Zheng.
Received: January 25, 2016; revised and accepted: February 24, 2016.
From the Department of Surgery (MA), Division of Surgical Oncology, Al-Azhar University Hospitals, Al-Azhar Faculty of Medicine, Cairo, Egypt, Department of Otolaryngology (MA, CTL, CJK, SFH), Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital at Linkou and Chang Gung University (CKT, FCW), Department of Epidemiology (HTC, CHL), Department of Medical Oncology (HMW), and Department of Radiation Oncology (KHF, JTC), Chang Gung Memorial Hospital at Linkou, Chang Gung University, Taoyuan, Taiwan, China.
Correspondence: Shiang-Fu Huang, Department of Otorhinolaryngology, Head and Neck Surgery, Chang Gung Memorial Hospital, at Linkou, Taiwan, R.O.C., No. 5, Fu-Shin Street, Kwei-Shan, Taoyuan, Taiwan 333, China (e-mail: shiangfu.huang@gmail.com).
This study was supported by grants NSC 102–2314-B-182A-081 and MOST 103–2314-B-182A-057-MY2 from the National Science Council, Executive Yuan, Taiwan, China.
Author contributions: MA and SFH designed and performed the experiments; SFH, HTC, and CHL analyzed the data; MA, FCW, CTL, HMW, KHF, CJK, JTC, and SFH contributed materials/methods; MA, CKT, and SFH prepared the manuscript.
The authors report no conflicts of interest.
Supplemental Digital Content is available for this article.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000003149

(*Medicine* 95(14):e3149)

Abbreviations: CRP = C-reactive protein, ECS = extracapsular spread, LND = lymph node density, OSCC = oral squamous cell carcinoma, SCC = squamous cell carcinoma, SCC-Ag = squamous cell carcinoma antigen.

INTRODUCTION

Lymph nodes metastasis, one of the most critical indicator in head and neck squamous cell carcinoma (SCC), affects patient survival.[1,2] Lymph node density (LND) has gained increasing attention in the last few years in evaluating the extent of nodal diseases in several human malignancies, including head and neck cancers.[3–7] LND, or lymph node ratio, is defined as the ratio of positive lymph nodes to the total number of excised lymph nodes.[8–10] Several studies have focused on the clinical relevance of LND in evaluating disease prognosis and its relationship with adverse outcomes in patients with oral and oropharyngeal SCC.[11–13] Furthermore, LND has a higher prognostic accuracy compared with tumor node metastasis (TNM) staging in nodal positive patients.[14] In a multicenter international study, Patel et al[15] reported that LND was superior to the conventional nodal staging system used by the American Joint Committee on Cancer (AJCC) in predicting oral squamous cell carcinoma (OSCC) outcomes because LND appeared to be a more precise predictor compared with the absolute number of metastatic lymph nodes; it considers not only the burden of nodal disease and tumor spread but also the extent of nodal dissection and surgical staging, thus alleviating certain limitations of technical performance and nodal sampling error.

Squamous cell carcinoma antigen (SCC-Ag) and C-reactive protein (CRP) have gained increasing attention in cancer research. Several OSCC-based studies have linked SCC-Ag with tumor aggressiveness, recurrence, and poor survival.[16–18] Similarly, studies have associated CRP, a sensitive marker of inflammation and tissue damage, with cancer aggression and patient survival.[19,20] In our previous study, concurrent elevated SCC-Ag and CRP levels exhibited significant potential as a biomarker for risk stratification in OSCC and as a predictor for lymph node metastasis, advanced tumor stage, and tumor recurrence.[21]

Although SCC-Ag, CRP, and LND may be correlated, their relationship remains unclear. Our primary aim is to identify any significant correlation between preoperative serum makers and postoperative pathological markers. Furthermore, we explore the possibility of risk stratification of LND by using preoperative serum SCC-Ag and CRP levels.

MATERIALS AND METHODS

Patients with OSCC

We identified 277 consecutive patients with primary OSCC between March 2008 and November 2013 from the

Chang Gung Memorial Hospital. All patients received radical surgery with curative intent as the primary modality of therapy. Patients with verrucous carcinoma or distant metastasis, patients who received preoperative chemoradiotherapy, patients with incomplete data, and patients lost to follow-up were excluded. All patients were followed from the time of cancer diagnosis until death or May 2015. The chart review comprised preoperative physical examination, complete blood count, chest radiographs, routine blood biochemistry, liver ultrasound, computed tomography or magnetic resonance imaging of the head and neck, and whole-body bone scan or positron emission tomography. Patient characteristics; tumor characteristics, such as tumor status, nodal status, histopathological features, overall stage, and oral site involved; and preoperative SCC-Ag and CRP levels were collected. The study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Linkou. All the patient records/information were anonymized and deidentified before analysis.

Treatment of OSCC

Patients were staged according to the TNM staging system proposed by the AJCC (2002 edition).[22] All patients had undergone radical excision of the tumor with clinical stage–based neck dissection after preoperative tumor survey. For clinically nodal negative and positive patients, ipsilateral supraomohyoid neck dissection, and ipsilateral modified radical neck dissection, respectively, were performed. If a lesion invaded deeply and crossed the midline, as observed in tongue cancer, bilateral neck dissection was performed. Primary tumors were excised with $\geq$1-cm safety margins (both peripheral and deep margins). Pathologic parameters, such as tumor cell differentiation, depth of tumor invasion, perineural invasion, lymphovascular invasion, skin or bone invasion, and lymph node extracapsular spread (ECS), were recorded. Patients with poor tumor differentiation, advanced tumor stage (T3 or T4), lymph node ECS, or $\geq$10-mm tumor depth received radiotherapy or concomitant chemoradiotherapy 4 to 8 weeks after the surgery.[21]

Measurement of CRP

To minimize intraindividual differences, preoperative serum CRP levels were evaluated at the time of tissue diagnosis before any medical intervention or antibiotic treatment was administered. Serum CRP levels were detected using an autoanalyzer (Hitachi 7600–210, Hitachi Medico, Tokyo, Japan). The cutoff point for serum CRP level was 5.0 mg/L, the internationally adopted cutoff point for inflammation.[19]

Measurement of SCC-Ag Levels

Serum SCC-Ag levels were measured at the time of tissue diagnosis using a commercially available chemiluminescent microparticle immunoassay (Abbott Japan Co, Ltd, Tokyo, Japan). The reference cutoff point for the serum SCC-Ag levels was 2.0 ng/mL, as established previously.[16,23]

Calculation of LND

LND was calculated as the ratio of the number of positive lymph nodes to the total number of excised lymph nodes. The cutoff point for the 2 groups was 0.06, as has been consistently used in several studies.[8,12] Patients were divided into 3 groups: reference group, no lymph node metastasis; group A, LND $<$ 0.06; and group B, LND $\geq$ 0.06.

Statistical Analysis

We performed univariate analysis (UVA) using the χ^2 test. UVA of survival differences was performed using the log-rank test. Multivariate analysis (MVA) of survival was performed using the Cox proportional hazard model for determining the prognostic value. Two-sided $P < 0.05$ was considered significant. The Statistical Package Software for the Social Sciences version 13.0 (SPSS, Inc, Chicago, IL) was used for the statistical analyses.

RESULTS

We identified 277 patients with OSCC, of whom 253 (91%) were men and 24 (8.7%) were women. The mean age at diagnosis was 51.9 years (range 27–84 years). The tongue was the most common primary site for OSCC (114 patients, 41.2%), followed by the buccal mucosa (106 patients, 38.3%). The tumor stage distribution was as follows: 62 (22.4%) in stage I, 46 (16.6%) in stage II, 37 (13.4%) in stage III, and 132 in stage IV (40.8% and 6.9% in stage IVa and IVb, respectively). Supplementary Table 1, http://links.lww.com/MD/A841 presents the clinicopathological characteristics of the patients.

Supplementary Table 2, http://links.lww.com/MD/A841, lists the significant associations of preoperative CRP, SCC-Ag levels, and the clinicopathological parameters. Concurrent elevated CRP and SCC-Ag serum levels correlated with the T status ($P < 0.001$), N stage ($P < 0.001$), lymph node metastasis with ECS ($P = 0.003$), tumor stage ($P < 0.001$), skin invasion ($P < 0.001$), bone invasion ($P < 0.001$), tumor depth $\geq$10 mm ($P < 0.001$), and perineural invasion ($P = 0.056$), whereas they did not correlate with the degree of tumor differentiation. Elevated CRP and SCC-Ag levels adversely influence disease-free survival (DFS) (Supplementary Figure 1A, http://links.lww.com/MD/A841, $P < 0.001$, hazard ratio [HR] = 1.478, 95% confidence interval [CI] = 1.231–1.774) and overall survival (OS) (Supplementary Figure 1B, http://links.lww.com/MD/A841, $P < 0.001$, HR = 1.658, 95% CI = 1.292–2.127).

Table 1 lists the associations between postoperative LND (cutoff point, 0.06) and clinicopathological parameters. LND was significantly correlated with pathological tumor status, nodal status, degree of differentiation, tumor stage, tumor depth, and perineural invasion ($P < 0.001$ for each parameter). In addition, LND was correlated with lymph node metastasis with ECS ($P < 0.001$), whereas no correlation was observed between LND and skin or bone invasion. Table 2 clarifies a significant correlation among LND and preoperative CRP ($\geq$5.0 mg/L) and SCC-Ag ($\geq$2.0 ng/mL) levels ($P = 0.006$).

The common prognostic covariates were age of disease onset, sex, nodal status with ECS, degree of differentiation, T status, LND, sole preoperative serum CRP and SCC-Ag levels, tumor depth, and combined preoperative serum CRP–SCC-Ag levels, all of which were collected to investigate their prognostic significance for 5-year DFS and OS through UVA (Table 3) and MVA (Table 4). UVA revealed that neither age nor sex was a significant predictor for survival outcome, whereas 5 histopathological parameters were significantly correlated with 5-year DFS and OS, tumor status ($P < 0.001$, for both), nodal status metastasis with ECS ($P < 0.001$, for both), degree of differentiation ($P < 0.001$, $P = 0.003$, respectively), tumor depth ($P = 0.005$, $P = 0.001$, respectively), and LND ($P < 0.001$, for both; Figure 1 A and B). In addition, preoperative serum CRP and SCC-Ag levels were significant predictors for survival, either combined ($P < 0.001$ for 5-year DFS and OS

TABLE 1. The Associations Between LND and Clinicopathologic Parameters (N = 277) in OSCC

	Reference Group, n (%)	0 < LND < 0.06, n (%)	LND ≥ 0.06, n (%)	P^*
Pathologic tumor status				
Early[†] (n = 163)	102 (67.1)	48 (61.5)	13 (27.7)	<0.001
Advanced[‡] (n = 114)	50 (32.9)	30 (38.5)	34 (72.3)	
Nodal status				
(−) metastasis, (−) ECS (n = 152)	152 (100.0)	0 (0.0)	0 (0.0)	<0.001
(+) metastasis, (−) ECS (n = 55)	0 (0.0)	46 (59.0)	9 (19.1)	
(+) metastasis, (+) ECS (n = 70)	0 (0.0)	32 (41.0)	38 (80.9)	
Differentiation				
Well (n = 90)	70 (46.1)	11 (14.1)	9 (19.1)	<0.001
Moderate (n = 151)	74 (48.7)	51 (65.4)	26 (55.3)	
Poor (n = 36)	8 (5.3)	16 (20.5)	12 (25.5)	
Tumor stage				
Early[§] (n = 102)	102 (67.1)	0 (0.0)	0 (0.0)	<0.001
Advanced[‖] (n = 175)	50 (32.9)	78 (100.0)	47 (100.0)	
Skin invasion				
No (n = 245)	137 (90.1)	71 (91.0)	37 (78.7)	0.071
Yes (n = 32)	15 (9.9)	7 (9.0)	10 (21.3)	
Bone invasion				
No (n = 218)	123 (80.9)	61 (78.2)	34 (72.3)	0.451
Yes (n = 59)	29 (19.1)	17 (21.8)	13 (27.7)	
Tumor depth ≥10 mm				
No (n = 123)	90 (59.2)	23 (29.5)	10 (21.3)	<0.001
Yes (n = 154)	62 (40.8)	55 (70.5)	37 (78.7)	
Perineural invasion				
No (n = 177)	118 (77.6)	44 (56.4)	15 (31.9)	<0.001
Yes (n = 100)	34 (22.4)	34 (43.6)	32 (68.1)	

ECS = extracapsular spread, LND = lymph node density, OSCC = oral squamous cell carcinoma.
[*] Chi-square trend test.
[†] T1–T2.
[‡] T3–T4.
[§] Stage I-II.
[‖] Stage III-IV.

for both) or separately ($P = 0.001$ for 5-year DFS and OS for CRP and $P = 0.001$ and 0.003 for 5-year DFS and OS, respectively, for SCC-Ag; Supplementary Figures 1A and 1B, http://links.lww.com/MD/A841). A significant association was observed between LND and the distant metastatic rate (Figure 1C, $P < 0.001$).

Moreover, we explored the role of SCC-Ag and CRP in the LND^+ group, which has previously exhibited a high risk of recurrence. Elevated SCC-Ag and CRP levels were associated with DFS (Supplementary Figure 2A, http://links.lww.com/MD/A841, $P = 0.010$) and OS (Supplementary Figure 2B, http://links.lww.com/MD/A841, $P = 0.003$).

DISCUSSION

SCC-Ag is a tumor-associated protein whose expression represents a visage of clinical aggressiveness and invasion. SCC-Ag was used as a biomarker for early diagnosis, disease progression, and recurrence detection or treatment response in several human SCC, including head and neck cancers.[17,24–27] The biological function of SCC-Ag remains unclear. SCC-Ag either affects the cell cycle by inhibiting apoptosis and promoting cancer cell survival or by increasing cell migration in response to stimulation by the epidermal growth factor.[28] Another contributing mechanism is the promotion of oncogenic transformation and induction of the NF-κB protein complex and interlukine-6 (IL-6) signaling, which in turns promotes protumorigenic inflammation and oncogenic transformation.[29]

The CRP is another potentially significant serum marker. CRP measurement is simple and easily accessible; furthermore, it is helpful in cancer care.[20] As reported in several human malignancies, the CRP has repeatedly been linked to aggressive tumor behavior and dismal survival in head and neck cancers, such as oral, pharyngolaryngeal, and nasopharyngeal SCC.[19,30–32] However, the exact mechanism through which the CRP is involved in tumorigenesis remains unclear. CRP has been reported as a stimulatory initiator of excessive cell proliferation and subsequent DNA damage through the promotion of chronic inflammation.[33] Other noteworthy evidence is the response of CRP to proinflammatory cytokines, such as IL-6, IL-8, and tumor necrosis factor in the tumor microenvironment, reflecting in part the subsequent host response and tumor cell lysis.[33–35] The host response theory is supported by the clinical application of CRP in monitoring the treatment response in cancer patients.[34]

We previously demonstrated the clinical relevance of using both markers concomitantly in risk stratification and prediction of local recurrence in patients with oral and

TABLE 2. The Associations Between Preoperative CRP, SCC-Ag, and LND (N = 277)

	CRP (−), SCC-Ag (−), n (%)	CRP (−), SCC-Ag (+), n (%)	CRP (+), SCC-Ag (−), n (%)	CRP (+), SCC-Ag (+), n (%)	P^*
LND					
Reference group (n = 152)	105 (63.6)	19 (47.5)	17 (43.6)	11 (33.3)	0.006
0 < LND <0.06 (n = 78)	42 (25.5)	12 (30.0)	13 (33.3)	11 (33.3)	
LND ≥ 0.06 (n = 47)	18 (10.9)	9 (22.5)	9 (23.1)	11 (33.3)	

CRP = C-reactive protein, LND = lymph node density; reference group = OSCC patients received neck dissection without lymph node metastasis, SCC-Ag = squamous cell carcinoma antigen. CRP (−): CRP level <5.0 mg/L; CRP (+): CRP level ≥5.0 mg/L; SCC-Ag (−): SCC-Ag <2.0 ng/mL; SCC-Ag (+): SCC-Ag ≥2.0 ng/ml.

* χ^2 test.

TABLE 3. Univariate Log-Rank Test of Prognostic Covariates in 277 Patients With Oral Cavity Squamous Cell Carcinoma Regarding Disease-free and Overall Survival

	Case Number	5-year Disease-free Survival Rate (%)	*P*, HR (95% CI)	5-year Overall Survival Rate (%)	*P*, HR (95% CI)
Age, y			0.190		0.742
<50	116	59.1	1	81.4	1
≥50	161	64.5	0.758 (0.501–1.147)	82.9	0.904 (0.495–1.650)
Sex			0.933		0.557
Female	24	66.4 (55 mns)	1	75.0 (55 mns)	1
Male	253	61.8	0.969 (0.468–2.005)	82.9	0.756 (0.298–1.922)
Nodal status			<0.001		<0.001
(−) metastasis, (−) ECS	152	74.9	1	93.9	1
(+) metastasis, (−) ECS	55	62.3	1.726 (0.951–3.131)	81.8	3.527 (1.360–9.143)
(+) metastasis, (+) ECS	70	32.6	4.852 (3.029–7.772)	53.8	9.917 (4.478–21.961)
Differentiation*			<0.001		0.003
Well/moderate	241	66.2	1	84.9	1
Poor	36	33.9	2.780 (1.702–4.540)	65.1	2.796 (1.408–5.552)
Tumor status			<0.001		0.001
Early*	163	70.1	1	88.2	1
Advanced†	114	50.5	2.326 (1.529–3.540)	72.9	2.739 (1.484–5.056)
Lymph node density			<0.001		<0.001
Reference group	152	74.9	1	93.9	1
0 < LND < 0.06	78	55.7	2.105 (1.256–3.526)	77.9	3.681 (1.525–8.884)
LND ≥ 0.06	47	28.9	5.885 (3.557–9.735)	48.3	13.495 (5.985–30.428)
SCC-Ag			0.001		0.003
<2 ng/mL	204	67.8	1	86.7	1
≥2 ng/mL	73	39.2	2.078 (1.344–3.213)	67.0	2.524 (1.375–4.632)
CRP			0.001		0.001
<5 mg/L	205	66.1	1	86.4	1
≥5 mg/L	72	51.1	2.096 (1.357–3.238)	70.2	2.776 (1.519–5.075)
Tumor depth			0.005		0.001
<10 mm	123	67.5	1	91.9	1
≥10 mm	154	57.4	1.889 (1.217–2.931)	74.0	3.489 (1.673–7.278)
SCC-Ag and CRP			<0.001		<0.001
SCC-Ag < 2 ng/mL, CRP < 5 mg/L	165	68.8	1	87.9	1
SCC-Ag ≥ 2 ng/mL, CRP < 5 mg/L	40	50.1	1.343 (0.722–2.496)	78.7	1.531 (0.608–3.857)
SCC-Ag < 2 ng/mL, CRP ≥ 5 mg/L	39	64.7	1.357 (0.730–2.520)	81.6	1.786 (0.746–4.277)
SCC-Ag ≥ 2 ng/mL, CRP ≥ 5 mg/L	33	32.8 (45 mns)	4.064 (2.348–7.033)	52.3	5.211 (2.494–10.888)

CI = confidence interval, CRP = C-reactive protein, ECS = extracapsular spread, HR = hazard ratio, LND = lymph node density, SCC-Ag = squamous cell carcinoma antigen.

* T1–T2.

† T3–T4.

TABLE 4. Multivariate Cox Regression Model of Prognostic Covariates in 277 Patients with Oral Cavity Squamous Cell Carcinoma Regarding DFS and OS

Characteristic	DFS *P*	DFS HR (95% CI)	OS *P*	OS HR (95% CI)
Age, y				
<50	0.296	1	0.847	1
≥50		0.793 (0.514–1.224)		0.940 (0.504–1.754)
Differentiation*				
Well/moderate	0.004	1	0.023	1
Poor		2.232 (1.298–3.839)		2.469 (1.134–5.376)
Tumor depth				
<10 mm	0.614	1	0.277	1
≥10 mm		0.861 (0.482–1.539)		1.696 (0.654–4.396)
Tumor status				
Early†	0.157	1	0.845	1
Advanced*		1.471 (0.862–2.508)		1.086 (0.476–2.481)
Nodal status				
(−) ECS	0.098	1	0.548	1
(+) ECS		1.671 (0.909–3.071)		1.293 (0.559–2.987)
Lymph node density				
Reference group	0.002	1	< 0.001	1
0 < LND < 0.06	0.459	1.283 (0.664–2.478)	0.170	2.093 (0.729–6.008)
LND ≥ 0.06	0.002	3.057 (1.503–6.217)	<0.001	7.481 (2.580–21.688)
SCC-Ag and CRP				
SCC-Ag < 2 ng/mL, CRP < 5 mg/L	0.027	1	0.095	1
SCC-Ag ≥ 2 ng/mL, CRP < 5 mg/L	0.893	0.955 (0.490–1.861)	0.722	0.834 (0.308–2.263)
SCC-Ag < 2 ng/mL, CRP ≥ 5 mg/L	0.788	1.095 (0.566–2.117)	0.740	1.171 (0.461–2.978)
SCC-Ag ≥ 2 ng/mL, CRP ≥ 5 mg/L	0.007	2.426 (1.277–4.608)	0.038	2.574 (1.052–6.296)

CI = confidence interval, CRP = C-reactive protein, DFS = disease-free survival, ECS = extracapsular spread, HR = hazard ratio, LND = lymph node density, OS = overall survival, SCC-Ag = squamous cell carcinoma antigen.
* T1–T2.
† T3–T4.

pharyngolaryngeal carcinoma.[21,30,36] According to the current results, a combination of serum markers was significant for 5-year DFS and OS according to UVA when evaluated in combination or separately. Furthermore, preoperative elevated marker levels were significantly related to pathological nodal status with ECS and positively correlated with LND, with a cutoff point of 0.06 ($P = 0.006$).

LND is a critical predictor for outcomes in oral, pharyngeal, hypopharyngeal, and laryngeal SCC.[6,7,13,37] A low LND strongly correlates with improved prognosis and reduction in the regional failure of the treatment.[7]Furthermore, owing to its simultaneous consideration of the extent of neck involvement (number of positive lymph nodes), nodal yield (total number of lymph nodes excised during dissection), and staging, LND was

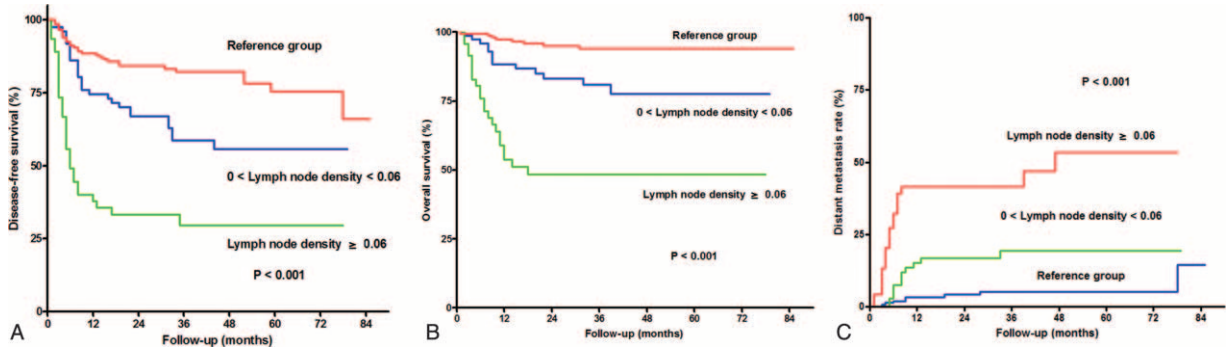

FIGURE 1. LND-related survival curves in 277 patients with OSCC. (A) The elevated LND group had significantly worse DFS compared with the nonelevated LND and nonlymph node metastatic reference group ($P < 0.001$). (B) The elevated LND group had significantly worse OS compared with the nonelevated LND and nonlymph node metastatic reference group ($P < 0.001$). (C) The elevated LND group had significantly higher risks of distant metastasis compared with the nonelevated LND and non-lymph node metastatic reference group ($P < 0.001$). DFS = disease-free survival, LND = lymph node density, OS = overall survival, OSCC = oral squamous cell carcinoma.

proven to exhibit a more significant prognostic value than does the current TNM staging system.[8,11,15] The current TNM staging system uses minimal detailed data regarding lymph node metastasis, namely, the number, size, and laterality of the positive lymph nodes.[38]

Several LND cutoff points have been proposed for predicting the survival and outcome of head and neck cancers.[8,11,12,14,15,39] In most studies, 0.06 was the cutoff point. Liao et al[11] used 0.16 for stage I–III neck dissection and 0.048 for stage I–V neck dissection as the cutoff points. This inter-study variation in the cutoff points is partly because of the nodal yield during neck dissection in different hospital settings. In addition, nodal yield is a valuable factor in patients with OSCC for discussing LND.[40,41] Nodal yield <18 has been associated with reduced OS, disease-specific survival (DSS), and DFS.[40] The average nodal yield per ipsilateral neck dissection in our study was 44.0 (range 10–141). Subjective significant variations of nodal yields may compromise the exact evaluation of the detection percentage and prognosis evaluation of LND. Patients can be stratified into low- and high-risk groups on the basis of LND measurement.[39] In the present study, patients with LND $\geq$ 0.06 had significantly worse OS and DSS. The patients in this high-risk group (patients with LND $\geq$ 0.06) can be stratified using the SCC-Ag and CRP serum levels.

Distant metastasis in OSCC is a relatively rare but clinically relevant event. Several studies have reported the relationship between the risk of distant metastasis and nodal status in head and neck SCC,[42,43] and early detection and treatment of the nodal disease may prevent distant metastases.[44] LND has been substantially associated with locoregional recurrence in OSCC,[6,21] and we have previously demonstrated the association of LND with the incidence of distant metastasis. The present study provides the evidence for the clinical relevance of LND in distant metastasis. During the follow-up period after the initial treatment, distant metastasis was diagnosed in 40 patients (14%, n = 277), which is in the reported range of distant metastases from 8% (n = 5019) to 17% (n = 769) in OSCC.[45] Because the lungs are the primary target organs for distant metastasis in OSCC,[46,47] more than half of the OSCC patients (in 21 patients) were metastasized to the lungs; in addition, metastasis to the lung concurrent with other sites was observed in 3 patients, skin metastasis was observed in 4 patients, and distant metastasis to different organs of the body was observed in the remaining patients. Among all the tested variables, LND $\geq$0.06 was the only independent factor when compared with the reference group (without lymph node metastasis) for distant metastasis according to MVA ($P = 0.001$, HR, 95% CI = 6.684 [2.279–19.607]). Interestingly, no significant associations were found between the combined elevated SCC-Ag and CRP levels (either together or individually) and the incidence of distant metastasis. However, we extrapolate that the combined elevated levels of serum markers may be indirectly related to distant metastasis through their significant correlation with LND.

LND, T status, depth of tumor invasion, degree of differentiation, perivascular invasion, and ECS all have predicted the survival and guided the adjuvant treatment of such patients.[11,15,48–52] Our results reveal a correlation between the elevated markers and adverse histopathological features of the tumor. We assume that by using clinically available serum markers, clinicians can predict unfavorable histopathological parameters before surgical procedures. Moreover, these elevated serum levels provide the clinicians information regarding the high likelihood of aggressive tumor behavior and the high probability of harboring occult nodal metastases; moreover, they facilitate postoperative treatment planning.

CONCLUSION

LND was correlated with the incidence of distant metastasis and DFS and OS in OSCC. Concurrent high preoperative SCC-Ag and CRP levels exhibited a linear correlation with LND and can be used as predictors for adverse tumor features. Preoperative elevated serum levels can be used to stratify patients for adjuvant treatment and must be considered complementary to the prognostic significance of LND.

ACKNOWLEDGMENTS

The authors thank all the members of the Cancer Center in Chang Gung Memorial Hospital, Linkou for their invaluable assistance.

REFERENCES

1. Shah JP, Candela FC, Poddar AK. The patterns of cervical lymph node metastases from squamous carcinoma of the oral cavity. *Cancer.* 1990;66:109–113.
2. Li XM, Di B, Shang YD, et al. Clinicopathologic features and prognostic factors of cervical lymph node metastasis in oral squamous cell carcinoma. *Ai Zheng.* 2005;24:208–212.
3. Ku JH, Kang M, Kim HS, et al. Lymph node density as a prognostic variable in node-positive bladder cancer: a meta-analysis. *BMC cancer.* 2015;15:447.
4. Li X, Liu Y, Cao B, et al. Metastatic lymph node ratio and prognosis of gastric cancer at different pT stages. *Hepatogastroenterology.* 2015;62:507–511.
5. Zhou D, Ye M, Bai Y, et al. Prognostic value of lymph node ratio in survival of patients with locally advanced rectal cancer. *Can J Surg.* 2015;58:237–244.
6. Prabhu RS, Hanasoge S, Magliocca KR, et al. Lymph node ratio influence on risk of head and neck cancer locoregional recurrence after initial surgical resection: Implications for adjuvant therapy. *Head Neck.* 2015;37:777–782.
7. Ebrahimi A, Clark JR, Zhang WJ, et al. Lymph node ratio as an independent prognostic factor in oral squamous cell carcinoma. *Head Neck.* 2011;33:1245–1251.
8. Gil Z, Carlson DL, Boyle JO, et al. Lymph node density is a significant predictor of outcome in patients with oral cancer. *Cancer.* 2009;115:5700–5710.
9. Attaallah W, Gunal O, Manukyan M, et al. Prognostic impact of the metastatic lymph node ratio on survival in rectal cancer. *Ann Coloproctol.* 2013;29:100–105.
10. Sayed SI, Sharma S, Rane P, et al. Can metastatic lymph node ratio (LNR) predict survival in oral cavity cancer patients? *J Surg Oncol.* 2013;108:256–263.
11. Liao CT, Hsueh C, Lee LY, et al. Neck dissection field and lymph node density predict prognosis in patients with oral cavity cancer and pathological node metastases treated with adjuvant therapy. *Oral Oncol.* 2012;48:329–336.
12. Ong W, Zhao R, Lui B, et al. Prognostic significance of lymph node density in squamous cell carcinoma of the tongue. *Hesad Neck.* 2015[Epub ahead of print].
13. Kunzel J, Psychogios G, Mantsopoulos K, et al. Lymph node ratio as a predictor of outcome in patients with oropharyngeal cancer. *Eur Arch Otorhinolaryngol.* 2014;271:1171–1180.

14. Kim SY, Nam SY, Choi SH, et al. Prognostic value of lymph node density in node-positive patients with oral squamous cell carcinoma. *Ann Surg Oncol.* 2011;18:2310–2317.
15. Patel SG, Amit M, Yen TC, et al. Lymph node density in oral cavity cancer: results of the International Consortium for Outcomes Research. *Br J Cancer.* 2013;109:2087–2095.
16. Lin WH, Chen IH, Wei FC, et al. Clinical significance of preoperative squamous cell carcinoma antigen in oral-cavity squamous cell carcinoma. *Laryngoscope.* 2011;121:971–977.
17. Imai R, Takenaka Y, Yasui T, et al. Prognostic significance of serum squamous cell carcinoma antigen in patients with head and neck cancer. *Acta Otolaryngol.* 2015;135:295–301.
18. Nakashima T, Yasumatsu R, Kuratomi Y, et al. Role of squamous cell carcinoma antigen 1 expression in the invasive potential of head and neck squamous cell carcinoma. *Head Neck.* 2006;28:24–30.
19. Chen HH, Chen IH, Liao CT, et al. Preoperative circulating C-reactive protein levels predict pathological aggressiveness in oral squamous cell carcinoma: a retrospective clinical study. *Clin Otolaryngol.* 2011;36:147–153.
20. Mahmoud FA, Rivera NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. *Curr Oncol Rep.* 2002;4:250–255.
21. Huang SF, Wei FC, Liao CT, et al. Risk stratification in oral cavity squamous cell carcinoma by preoperative CRP and SCC antigen levels. *Ann Surg Oncol.* 2012;19:3856–3864.
22. Greene FL, Page DL, Fleming ID, et al. American Joint Committee on Cancer: AJCC cancer staging manual. 6th ed. New York: Springer-Verlag; 2002.
23. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer.* 1977;40:1621–1628.
24. Wishnow KI, Johnson DE, Fritsche HA. Squamous cell carcinoma antigen in genitourinary tumors. *Int J Biol Markers.* 1989;4:226–228.
25. Zhang J, Zhu Z, Liu Y, et al. Diagnostic value of multiple tumor markers for patients with esophageal carcinoma. *PLoS One.* 2015;10:e0116951.
26. Yazigi R, Munoz AK, Richardson B, et al. Correlation of squamous cell carcinoma antigen levels and treatment response in cervical cancer. *Gynecol Oncol.* 1991;41:135–138.
27. Catanzaro JM, Guerriero JL, Liu J, et al. Elevated expression of squamous cell carcinoma antigen (SCCA) is associated with human breast carcinoma. *PLoS One.* 2011;6:e19096.
28. Takeda A, Kajiya A, Iwasawa A, et al. Aberrant expression of serpin squamous cell carcinoma antigen 2 in human tumor tissues and cell lines: evidence of protection from tumor necrosis factor-mediated apoptosis. *Biol Chem.* 2002;383:1231–1236.
29. Sheshadri N, Catanzaro JM, Bott AJ, et al. SCCA1/SERPINB3 promotes oncogenesis and epithelial-mesenchymal transition via the unfolded protein response and IL6 signaling. *Cancer Res.* 2014;74:6318–6329.
30. 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:e55327.
31. Tang LQ, Hu DP, Chen QY, et al. Elevated high-sensitivity C-reactive protein levels predict decreased survival for nasopharyngeal carcinoma patients in the intensity-modulated radiotherapy era. *PLoS One.* 2015;10:e0122965.
32. Andersson BA, Lewin F, Lundgren J, et al. Plasma tumor necrosis factor-alpha and C-reactive protein as biomarker for survival in head and neck squamous cell carcinoma. *J Cancer Res Clin Oncol.* 2014;140:515–519.
33. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420:860–867.
34. Wang CS, Sun CF. C-reactive protein and malignancy: clinico-pathological association and therapeutic implication. *Chang Gung Med J.* 2009;32:471–482.
35. Black S, Kushner I, Samols D. C-reactive Protein. *J Biol Chem.* 2004;279:48487–94840.
36. Chen IH, Liao CT, Wang HM, et al. Using SCC antigen and CRP levels as prognostic biomarkers in recurrent oral cavity squamous cell carcinoma. *PLoS One.* 2014;9:e103265.
37. Wang YL, Feng SH, Zhu J, et al. Impact of lymph node ratio on the survival of patients with hypopharyngeal squamous cell carcinoma: a population-based analysis. *PLoS One.* 2013;8:e56613.
38. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol.* 2010;17:1471–1474.
39. Shrime MG, Ma C, Gullane PJ, et al. Impact of nodal ratio on survival in squamous cell carcinoma of the oral cavity. *Head Neck.* 2009;31:1129–1136.
40. Ebrahimi A, Zhang WJ, Gao K, et al. Nodal yield and survival in oral squamous cancer: Defining the standard of care. *Cancer.* 2011;117:2917–2925.
41. Feng Z, Qin LZ, Huang X, et al. Nodal yield: is it a prognostic factor for head and neck squamous cell carcinoma? *J Oral Maxillofac Surg.* 2015;73:1851–1859.
42. Kuperman DI, Auethavekiat V, Adkins DR, et al. Squamous cell cancer of the head and neck with distant metastasis at presentation. *Head Neck.* 2011;33:714–718.
43. Leon X, Quer M, Orus C, et al. Distant metastases in head and neck cancer patients who achieved loco-regional control. *Head Neck.* 2000;22:680–686.
44. Gray L, Woolgar J, Brown J. A functional map of cervical metastases from oral squamous cell carcinoma. *Acta Otolaryngol.* 2000;120:885–890.
45. Betka J. Distant metastases from lip and oral cavity cancer. *ORL J Otorhinolaryngol Relat Spec.* 2001;63:217–221.
46. Hasegawa T, Tanakura M, Takeda D, et al. Risk factors associated with distant metastasis in patients with oral squamous cell carcinoma. *Otolaryngol Head Neck Surg.* 2015;152:1053–1560.
47. Ferlito A, Shaha AR, Silver CE, et al. Incidence and sites of distant metastases from head and neck cancer. *ORL J Otorhinolaryngol Relat Spec.* 2001;63:202–207.
48. Moore C, Flynn MB, Greenberg RA. Evaluation of size in prognosis of oral cancer. *Cancer.* 1986;58:158–162.
49. Sawazaki-Calone I, Rangel A, Bueno AG, et al. The prognostic value of histopathological grading systems in oral squamous cell carcinomas. *Oral Dis.* 2015;21:755–761.
50. Fang KH, Kao HK, Cheng MH, et al. Histological differentiation of primary oral squamous cell carcinomas in an area of betel quid chewing prevalence. *Otolaryngol Head Neck Surg.* 2009;141:743–749.
51. Carrillo JF, Carrillo LC, Cano A, et al. Retrospective cohort study of prognostic factors in patients with oral cavity and oropharyngeal squamous cell carcinoma. *Head Neck.* 2016;38:536–541.
52. de Juan J, Garcia J, Lopez M, et al. Inclusion of extracapsular spread in the pTNM classification system: a proposal for patients with head and neck carcinoma. *JAMA Otolaryngol Head Neck Surg.* 2013;139:483–488.