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Retrospective Study

Risk factors and a predictive nomogram for lymph node metastasis in superficial esophageal squamous cell carcinoma

Wang J *et al*. Lymph node metastasis of superficial ESCC

Jin Wang, Xian Zhang, Tao Gan, Ni-Ni Rao, Kai Deng, Jin-Lin Yang

Abstract

BACKGROUND

Superficial esophageal squamous cell carcinoma (ESCC) is defined as cancer infiltrating the mucosa and submucosa, regardless of regional lymph node metastasis (LNM). Endoscopic resection of superficial ESCC is suitable for lesions that have no or low risk of LNM. Patients with a high risk of LNM always need further treatment after endoscopic resection. Therefore, accurately assessing the risk of LNM is critical for additional treatment options.

AIM

To analyze risk factors for LNM and develop a nomogram to predict LNM risk in superficial ESCC patients.

METHODS

Clinical and pathological data of superficial ESCC patients undergoing esophagectomy from January 1, 2009, to January 31, 2016, were collected. Logistic regression analysis was used to predict LNM risk factors, and a nomogram was developed based on risk factors derived from multivariate logistic regression analysis. The receiver operating characteristic (ROC) curve was used to obtain the accuracy of the nomogram model.

RESULTS

A total of 4660 patients with esophageal cancer underwent esophagectomy. Of these, 474 superficial ESCC patients were enrolled in the final analysis, with 322 patients in the training set and 142 patients in the validation set. The prevalence of LNM was 3.29% for intramucosal cancer and increased to 26.40% for submucosal cancer. Multivariate logistic analysis showed that tumor size, invasive depth, tumor differentiation, infiltrative growth pattern, tumor budding, and lymphovascular invasion were significantly correlated with LNM. A nomogram using these six variables showed good

discrimination with an area under the ROC curve of 0.789 (95%CI: 0.737-0.841) in the training set and 0.827 (95%CI: 0.755-0.899) in the validation set.

CONCLUSION

We developed a useful nomogram model to predict LNM risk for superficial ESCC patients which will facilitate additional decision-making in treating patients who undergo endoscopic resection.

Key Words: Superficial esophageal squamous cell carcinoma; Lymph node metastasis; Risk factors; Nomogram; Predictive model

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Core Tip: This is a retrospective study to identify risk factors for lymph node metastasis (LNM) in superficial esophageal squamous cell carcinoma (ESCC) and to develop a nomogram model for predicting LNM. A total of 474 superficial ESCC patients who underwent esophagectomy were enrolled. Multivariate logistic analysis showed that tumor size, invasive depth, tumor differentiation, infiltrative growth pattern, tumor budding, and lymphovascular invasion were significantly correlated with LNM. A predictive nomogram using these six variables showed good performance and will facilitate the treatment choice for superficial ESCC patients.

INTRODUCTION

Superficial esophageal squamous cell carcinoma (ESCC) is defined as esophageal cancerous lesions infiltrating the mucosa and submucosa, regardless of lymph node metastasis (LNM)[1]. Endoscopic resection has been the first treatment choice for

superficial ESCC patients with no or low risk of LNM and has an *en* bloc rate of more than 90%[2,3]. However, additional treatment is recommended for patients with high risk of LNM, especially for patients with positive lymphovascular invasion (LVI) and positive vertical margins[4]. Assessing pathological characteristics after endoscopic resection and predicting the risk of LNM is critical for additional treatment strategies.

Recent studies have established several predictive models to identify LNM risk for superficial ESCC patients[5-8]. However, some limitations existed in these models. For example, some studies did not incorporate critical factors, such as tumor budding and tumor infiltrative growth (INF) pattern, into LNM risk prediction. In some studies, the submucosa was considered as an entire layer[5,6] and the depth was not classified as submucosa 1 (SM1), SM2 or more[7,8]. Therefore, we aimed to establish a nomogram predictive model based on comprehensive pathological features obtained from esophagectomy to improve the predictive performance of such models.

MATERIALS AND METHODS

Patients

Patients who underwent esophagectomy at West China Hospital of Sichuan University from January 1, 2009, to January 31, 2016, were enrolled. The inclusion criteria were as follows: (1) Histopathological diagnosis of esophageal cancer; and (2) patients who received esophagectomy. The exclusion criteria were as follows: (1) Not pT1 stage tumor; (2) the histologic type was not squamous cell carcinoma; (3) number of dissected lymph nodes < 12; (4) history of previous malignancies; (5) incomplete clinical data; and (6) lesions with low-grade intraepithelial neoplasia or high-grade intraepithelial neoplasia. This retrospective study was approved by the Institutional Review Board of West China Hospital of Sichuan University (No. 2015-159). Informed consent was signed before the surgery.

Data collection

General clinical and endoscopic features, such as age, sex, tumor location, and endoscopic type, were retrospectively collected. Tumor location was defined as (1) upper: 15 to 24 cm from the incisors; (2) middle: 24 to 32 cm from the incisors; and (3) lower: 32 cm from the incisors to the cardia. Endoscopic types were identified according to the Paris classification criteria for superficial tumors[9]. The pathologic diagnosis was independently confirmed by two experienced pathologists. If the diagnosis is inconsistent, a third expert pathologist will re-examine the specimen, and the final diagnosis will be made when two or more pathologists agree on the diagnosis.

Data regarding pathological characteristics of specimens, such as tumor size, invasion depth, differentiation grade, INF pattern, tumor budding, LVI, and number of dissected lymph nodes, were collected. The vertical invasion depth of submucosal invasion was measured from the muscularis mucosae according to the Japanese guidelines[10]. The invasion depth was classified into four layers: Muscularis mucosae (MM), upper third of submucosa (SM1), middle third of submucosa (SM2) and lower third of submucosa (SM3) according to the Japanese Classification of Esophageal Cancer[10]. The differentiation grade was grouped as well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3)[11]. The tumor INF pattern was carefully observed and classified into three groups according to previous reports[10,12]: INF-a (expansive type, expansive growth of tumor nests downward continuously from the epithelium as a whole), INF-b (intermediate type between INF-a and INF-c), and INF-c (infiltrative type, tumor infiltrates with a way of single cell or small tumor nests, or trabecular arrangement of tumor cells on the leading edge of the tumor). Tumor budding is defined as a single tumor cell or a small tumor nest consisting of up to 4 cells at the front of the tumor invasion[13]. In this study, tumor budding was assessed on hematoxylin and eosin-stained slides at the front of tumor invasion. Tumor budding was categorized into three types: no budding, low-grade tumor budding (1 to 4 budding foci at a 20 × objective lens) and high-grade tumor budding (≥ 5 budding foci at a 20 × objective lens) according to a previous study[14]. For LVI, two experienced pathologists observed the same specimen to improve diagnostic accuracy.

Statistical analysis

Continuous variables are presented as mean ± SD and are compared with a *t* test in the case of a normal distribution. Categorical data are presented as percentages and are compared with the chi-square test or Fisher's exact test. All variables associated with LNM at a significant level were enrolled in the stepwise multivariate logistic analysis. All data were statistically analyzed by SPSS 22.0 software (IBM SPSS, Chicago, IL, United States). *P*-values < 0.05 were considered statistically significant.

R software (version 4.1.3) with the rms package was used to formulate a predictive nomogram using variables derived from multivariate logistic analysis. The pROC package was used to formulate the receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was used to evaluate the predictive performance of this nomogram as previously reported[15]. The nomogram can convert each regression variable to a scale of 0-100 points based on the regression coefficient. Finally, the predicted probabilities were derived from the total points obtained from each independent variable.

RESULTS

Clinicopathologic characteristics

In total, 4660 esophageal cancer patients underwent esophagectomy from January 1, 2009, to January 31, 2016, of which, 474 superficial ESCC patients were enrolled in the final analysis (Figure 1). The clinicopathologic characteristics of enrolled patients are presented in Table 1. Most of the superficial ESCC patients were male (77.4%), the average age was 60 (range, 38-84) years, and the average tumor size was 23 (range, 3-73) mm. Most tumors (63.9%) were located in the middle third of the esophagus. According to the endoscopic appearance, 309 (65.2%) tumors presented as flat lesions. Regarding invasion depth, 152 (32.1%) patients had intramucosal cancer, 80 (16.9%) patients had SM1 cancer, and 242 (51.0%) patients had tumors deeper than SM1. Regarding tumor differentiation, 103 (21.7%) patients, 279 (58.9%) patients, and 92 (19.4%) patients had

well differentiated, moderately differentiated, and poorly differentiated tumors, respectively. INF-b was the most common INF pattern, with 232 (48.9%) cases reported as such. The total tumor budding rate was 13.5% (64/474), and the LVI rate was 5.7% (27/474). Overall, 90 of the 474 (16.48%) patients had lymph node metastasis (LNM), and the LNM rate was 3.29% (5/152) in T1a tumors and 26.40% (85/322) in T1b tumors. The average number of dissected lymph nodes was 18.0 (range, 12-53) (Table 1).

Risk factors for LNM

The 474 enrolled patients were randomly grouped into a training set and a validation set at a ratio of 7:3. Comparisons of clinicopathological characteristics between the LNM+ and LNM- groups are presented in Table 2. Variables such as tumor size, invasion depth, tumor differentiation, INF pattern, tumor budding and LVI were significantly associated with LNM in both the training set and validation set according to the univariate analysis (Table 2). Furthermore, multivariate logistic regression analysis also showed that tumor size, invasion depth, tumor differentiation, INF pattern, tumor budding, and LVI were independent risk factors for LNM (Table 3).

Development and validation of the nomogram

Subsequently, a nomogram was developed based on six independent risk factors derived from the multivariate analysis (Figure 2). The point of each factor was proportional to its own β-coefficient resulted from logistic regression. Finally, the total points of each factor were added and visually corresponded to a predictive value for LNM. The ROC curve showed that this nomogram had good predictive performance both in the training set and in the validation set, with AUCs of 0.789 (95%CI: 0.737-0.841) and 0.827 (95%CI: 0.755-0.899), respectively (Figure 3).

DISCUSSION

Endoscopic resection has become one of the preferred treatment methods for superficial ESCC. Compared to surgery, it has fewer complications and a shorter recovery time[16].

Guidelines and studies have indicated that endoscopic submucosal dissection (ESD) and endoscopic submucosal tunnel dissection (ESTD) can be used to treat lesions limited to the muscularis mucosa and submucosal lesions with invasion depths ≤ 200 μm, which have no or an extremely low risk of lymph node metastasis[2,17-19]. The *en bloc* resection rate of ESD for superficial ESCC is 98.2%-100%, and the curative resection rate is 78.2%-96.1%[17-19]. However, patients with a high risk of LNM or noncurative endoscopic resection always need further treatment[4]. Therefore, summarizing post-ESD pathological characteristics and identifying risk factors for predicting LNM are critical to guide post-ESD treatment. In this study, we enrolled superficial ESCC patients who underwent esophagectomy and lymph node dissection and collected detailed pathological information, such as tumor budding and tumor infiltrative growth pattern, to comprehensively analyze and identify the risk factors for LNM, providing favorable evidence for post-ESD treatment decisions.

Our findings in this study indicated that superficial ESCC patients with positive LNM were more likely to have larger tumors, deeper invasion, poorer differentiation, more INF-c infiltrative patterns, more high-grade tumor budding, and more positive LVI both in both the training and validation sets. Some previous studies have likewise indicated that tumor size is positively correlated with LNM risk[5-8]. Ruan *et al*[8] found that tumor size > 2 cm was an independent risk factor for LNM in superficial ESCC. Our results showed that patients with tumor size > 3 cm had a higher risk of LNM (Figure 2). In another study, it was reported that a 1-cm increase in esophageal tumor length increased the LNM risk by 3.55 times[20]. Therefore, it is necessary to measure the area of cancer cells after the ESD procedure. If possible, pathological recovery should be performed to determine tumor size after the ESD procedure since it is crucial for predicting LNM risk.

In addition, we found that tumor invasion depth is associated with LNM risk. It was reported that the LNM rates of T1a and T1b superficial ESCC were 6.2%-8.0% and 20.0%-29.3%, respectively[21-23]. Similarly, in our study, the LNM rate in T1a tumor was 3.29% (5/152) and increased to 26.40% (85/322) in T1b tumor. Further multivariate

analysis showed that an invasion depth deeper than SM1 (OR: 15.517, 95%CI: 4.707-51.158) is an independent risk factor for LNM, which has been confirmed by other studies[5,6]. The esophageal submucosa is an area rich in lymphovascular network, and once intruded into the submucosa, tumor cells are more likely to infiltrate vascular systems[24,25]. In our study, LVI positivity (OR: 3.767, 95%CI: 1.422-9.979) was also an independent risk factor for LNM. Therefore, additional treatment, such as surgery or chemoradiotherapy, should be recommended for post-ESD patients with submucosal invasion deeper than 200 μm and positive LVI.

It has been well established that the grade of tumor budding is positively correlated with the rate of LNM in solid cancers, including gastrointestinal cancers[26-28]. We found that high-grade tumor budding (OR: 3.905, 95%CI: 1.387-10.995) was positively correlated with LNM risk in superficial ESCC. Similarly, Li *et al*[29] checked tumor budding in pT1b ESCC by using Pan-CK immunohistochemical (IHC) staining and found that the tumor budding level is an excellent predictor of LNM and patient survival time. However, there is no gold standard for differentiating the threshold value of tumor budding in superficial ESCC specimens, and a clear and standardized method for distinguishing and reporting tumor budding in superficial ESCC is urgently needed. In addition, tumor budding status in post-ESD specimens should be carefully assessed and reported.

The Japanese guidelines recommend that tumor infiltrative growth patterns should be reported in esophageal cancer[10]. Some studies have identified infiltrative type c (INF-c) as associated with deep tumor invasion, poor tumor differentiation, and a high risk of LNM[12,30,31]. In our study, multivariate logistic regression identified INF-b (OR: 3.205; 95%CI: 1.637-6.274), INF-c (OR: 4.121, 95%CI: 1.566-10.843) and poor differentiation (OR: 3.670, 95%CI: 1.465-9.198) as independent risk factors for LNM. Invasion of surrounding tissue by cancer cells is a key step in tumor progression and metastasis[32]. As the tumor grows, the morphology and behaviour of cancer cells at the tumor front undergo epithelial mesenchymal transition, detaching from the tumor body and infiltrating deep into the submucosa or even deeper[30]. Poorly differentiated

tumors are more likely to have LVI and LNM in superficial ESCC, as previously reported[6,8]. Therefore, a detailed assessment of tumor differentiation, INF pattern, tumor budding, and LVI is critical for predicting LNM risk in post-ESD patients.

Overall, we analyzed the risk factors for LNM in superficial ESCC patients by evaluating detailed pathological characteristics and developed a nomogram by incorporating six variables, including tumor size, invasion depth, tumor differentiation, tumor budding, tumor infiltrative growth pattern and LVI. This nomogram showed good predictive performance, with an AUC of 0.789 (95%CI: 0.737-0.841) in the training set and 0.827 (95%CI: 0.755-0.899) in the validation set. Although the data for this nomogram come from surgical specimens, we believe it is also applicable to post-ESD patients, as all six enrolled predictors can be easily obtained from post-ESD specimens. The use of this predictive nomogram will facilitate the assessment of LNM, thus providing references for guiding post-ESD treatment. However, this is a single-center retrospective study. More multicenter studies are needed to further confirm the reliability of the nomogram.

In addition, this study has some limitations. First, this is a retrospective study, and bias in case selection cannot be avoided. Second, differences in surgical procedures and chronological differences in pathological diagnostic criteria may affect the consistency of the results. D2-40 and CD34 IHC staining were not used in LVI diagnoses in this study, which would lead to underestimating the positivity of LVI. Finally, the LNM rate of superficial ESCC in this study cannot accurately represent the overall LNM rate because patients who underwent ESD were excluded because their postoperative LNM rate could not be calculated.

CONCLUSION

In conclusion, we identified the risk factors for LNM in superficial ESCC patients and developed a useful nomogram for predicting LNM risk by integrating all significant risk factors. This nomogram model will facilitate decision-making regarding additional treatment options in post-ESD patients.

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