



ORIGINAL ARTICLE

Prognostic significance of perineural invasion in laryngeal cancer

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Abstract

Introduction: Perineural invasion (PNI) may be an independent risk factor for lymph node metastasis in laryngeal cancer, impacting on decreased survival, increased rates of locoregional recurrence, and shortening the period for recurrence.

Objective: To evaluate the prognostic significance of PNI in laryngeal cancer. **Materials and Methods:** This is a retrospective study, with review of anatomopathological material of patients with laryngeal cancer surgically treated from March 2009 to May 2016. The presence of PNI is related to the oncological results. **Results:** Of the 69 patients studied, four presented PNI in the review of the slides, one of which presented local recurrence and two, regional relapse. **Conclusion:** The finding of PNI was 5.8%.

Keywords: laryngeal neoplasms; perineural invasion; prognostic factors; surgery.

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Introduction

Several studies have related perineural invasion (PNI) and other histopathological parameters as prognostic factors for laryngeal cancer, such as tumor thickness, concomitant vascular invasion, degree of cell differentiation, inflammatory reaction, depth of invasion and tumor volume¹⁻⁴. After the definition of Batsakis, in 1985, PNI was considered an important pathway for metastatic dissemination, and a nerve invasion by tumor cells, either around or inside the tumor, was defined as affecting the three layers (epineurium, perineurium and endoneurium)⁵. In order to initiate prediction of prognosis, IP was also included as a parameter in classification systems⁶ and authors recommend the investigation of species of laryngectomy for IP since then⁷. Its presence in microscopic studies of tumors of the larynx necessitated its inclusion as a criterion for the choice of treatment modality, especially for adjuvant radiotherapy, a mode of treatment which began to be performed under clinical conditions with microscopic foci of residual disease⁸. Lymph node metastasis appears to be the most important factor determining survival in patients with squamous cell carcinoma of the larynx⁹. PNI is a predictive indicator of metastatic cervical disease, whether hidden or not¹⁰. The incidence of PNI is more frequent in patients with locoregional recurrence and this may also be a recommendation for neck dissection¹¹.

The objective of this study is to evaluate the occurrence and prognostic significance of PNI in laryngeal squamous cell carcinoma.

Methods

This is a retrospective clinical-pathological study of patients with at least 24 months of follow-up, negative surgical margins and absence of a second synchronic or metachronous tumor. Patients were submitted to surgical treatment (partial or total laryngectomy, according to oncologic criteria based on staging), followed or not by adjuvant treatment (radiotherapy or chemo-radiotherapy).

Thus, medical records of 84 consecutive patients who underwent laryngeal squamous cell carcinoma were retrospectively evaluated between 2000 and 2012. The following aspects were evaluated: gender (male or female); age at the time of operation (in years); (semi-quantitative evaluation of packets and year-doses), subsite of the tumor (supraglottic, glottis or transglottic) and staging of the tumor. Patients were investigated for PNI and positive cases were reassessed for: size of extension, size of the largest nerve involved, distance from PNI to tumor border and classification of invasion pattern (intratumoral and extratumoral).

A study was conducted to determine whether histopathological evidence of small nerve PNI (<1 mm in diameter) affects the progression of patients treated with laryngeal squamous cell carcinoma. The anatomopathological slides and respective paraffin blocks of the resected tumors were recovered and reviewed by the same pathologist. The color of them all was by H-E. The PNI, defined as tumor cells touching or invading a small nerve, was examined. The measurement on the nerve diameter was performed by optical micrometry. Whether the axons were invaded by the tumor and whether the PNI occurred in the context of a tumor mass or peripherally to the tumor was found.

The outcomes were: local, regional and distant recurrence, regional staging (presence of lymph node metastases), and overall disease-free survival.

For statistical analysis, chi-square test was used to compare the categorical variables. The Mantel-Haenszel method was used to compare PNI with recurrence adjusted by tumor subsite.

Results

Of the 84 patients diagnosed with biopsy-proven laryngeal squamous cell carcinoma, 69 were included because they presented complete data, and of these 4 were PNI positive. Fifteen patients were excluded due to incomplete clinical information. Thus, the patients were divided into negative PNI, adding 65 and those positive PNI, adding 4.

For the negative PNI group, 56 were male and the mean age was 63.5 years. Forty-three were smokers and 15 were alcoholics. The most frequent tumor site was the glottis, followed by supraglottic. Regarding staging of the primary tumor (T), 28 were T1, 14 T2, 18 T3, and 5 T4. As for lymph node extension (N), 55 were N0, N1 4, N2a 2, and N2b 4. Thirty-four did not undergo any

adjuvant treatment, 16 underwent radiation therapy, 14 underwent radiation therapy associated with chemotherapy. Ten presented local recurrence and 7 presented regional recurrence. While there was 1 loss of follow-up, 51 were alive without evidence of disease, and 13 died from cancer. The mean time of follow-up was 33.8 months (Table 1).

The following patients had positive PNI:

- Patient 1, male, 59 years old, smoker and alcoholic, presented tumor of supraglottic site, stage T3N2c and performed adjuvant radiotherapy. There was a regional recurrence in 2 months and died 8 months after treatment. The size of the perineural invasion was 0.1 mm, distance between the PNI focus at the tumor border 0.1 mm, size of the largest nerve involved 0.2 mm, and the invasion pattern was extratumoral;
- Patient 2, male, 53 years old, smoker, presented a glottic site tumor, stage T1N0, and did not perform adjuvant therapy. There was no relapse and was alive without evidence of disease with a follow-up time of 26 months. The size of the invasion was 0.1 mm, distance between the PNI focus to the 0.6 mm tumor border, size of the largest nerve involved 0.2 mm, with pattern of extratumoral invasion;
- Patient 3, male, 58 years old, with tumor of glottic extension, stage T4N1, performed adjuvant radiotherapy. There was a local recurrence at 24 months and a regional recurrence at 23 months and was dead from the disease at 59 months. The size of the invasion was measured in 0.1 mm, distance between the PNI focus to the 0.4 mm tumor border, size of the largest nerve involved 0.2 mm, of extratumoral pattern;

Table 1. Clinical data of patients with negative PNI.

Negative Perineural Invasion (65)					
		N			N
Gender	Male	56	Adjuvant treatment	None	34
	Female	9		Radiotherapy	16
Smokers		43		Radiotherapy + Chemotherapy	14
Alcoholics		15	Relapse	Local	10
Age (average in years)		63.5		Regional	7
T	T1	28	N	N0	55
	T2	14		N1	4
	T3	18		N2a	2
	T4	5		N2b	4
Condition	Loss of follow-up	1	Time of follow-up (average in months)		33.8
	Alive without disease	51			
	Death by cancer	13			

- Patient 4, male, 65 years old, smoker and alcoholic, with transglottic tumor, stage T3N0, performed adjuvant radiotherapy and chemotherapy. There was regional recurrence in 14 months and cancer death after 16 months. The size of the invasion was 0.01 mm, distance between the PNI focus to the tumor border 0.1 mm, size of the largest nerve involved 0.2 mm, of extratumoral pattern.

Discussion

PNI of a tumor has been associated with poor prognosis in head and neck neoplasms because tumor cells can propagate longitudinally and radially through the perineural space beyond the surgical margins^{9,10}. It is a histopathological finding that indicates aggressive behavior of the tumor. It is a tumor-propagating route in which tumor cells enter the perineural space in order to spread proximally and distally along the nerve fiber. As soon as detected, tumor cells may be present beyond the margins of surgical control. Apparently, there is a negative impact on the prognosis that decreases disease-free survival, in addition to overall patient survival⁷.

A considerable number of histopathological features have been proposed as key questions in the prediction of recurrence and survival in patients with laryngeal cancer, including PNI. However, when analyzed separately, these factors produce contradictory conclusions⁹.

Several theories have been proposed in order to understand the exact mechanism of tumor propagation. Tumor cells are known to propagate along the connective tissue or the endoneural plane. The expression of the neural cell adhesion molecule (N-CAM) has been shown to be positively related to perineural spread in a variety of tumors¹². Once the tumor cells detach themselves from the main tumor mass and invade a nerve, they can spread proximally and distally. Involvement often begins near the terminal nerves and spreads proximally and progressively to involve larger trunks. Involvement of some genes with perineural invasion in head and neck cancer, such as neural cell adhesion molecules, has already been reported¹³. The association between up-regulation of cortactin in deep invasive frontal cells and perineural infiltration suggests that both the region and the putative marker can be considered a classification system in laryngeal carcinomas¹⁴.

PNI is difficult to evaluate in the preoperative because specimens of diagnostic biopsy are very small. It is likely that perineural inflammation and nerve fiber degeneration indicate the presence of perineural invasion in the area. Tumor cells in the perineural space may demonstrate only subtle signs of atypia and may be misinterpreted as benign. It has been established that tumor cells tend to focus on the periphery of the nerve (perineurium). Blood supply in this area is generally poor, while the space is relatively hypoxic and can lead to low radiosensitivity. This may explain the failure of previous radiotherapy. Therefore, in these cases, the borders of surgical resection need to be free, both of mucosa and nerve lining. PNI of smaller nerves remains a potential pathway for squamous cell carcinoma of the larynx and hypopharynx. The extension of tumor cells in the major nerves in laryngeal or pyriform breast carcinomas has been shown to be unlikely. The reduced number of upper and lower infiltrated laryngeal nerves (in only one case

out of 219) demonstrated that this mechanism of tumor extension was not applicable in cases of laryngeal or hypopharyngeal cancer¹³.

PNI has demonstrated prognostic predictive power in patients with head and neck carcinoma, in addition to being associated with a higher risk of locoregional recurrence, however, without a higher rate of distant metastases. In a study of 180 patients undergoing total pharyngolaryngectomy, PNI was an independent prognostic factor for univariate analysis¹⁵. Even in performing a partial laryngectomy, the finding of PNI alone may be considered as an indication for adjuvant radiotherapy¹⁶.

PNI is related to end-stage disease. There is a strong trend towards neural invasion in end stage carcinoma. This may explain the low PNI index found in our series, which had a grand number of early tumors. Although there is controversy, the preponderance of evidence in the literature suggests that PNI is a significant prognostic indicator in the ability to spread laryngeal cancer to cervical lymph nodes and therefore should be strongly considered when considering cervical dissection or using adjunctive treatment.

Conclusion

PNI was found in 5.8% of the cases, and it was not possible to establish a statistical correlation with oncological results.

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