

## Prognostic Value of Tumor-Infiltrating Lymphocytes for Patients With Head and Neck Squamous Cell Carcinoma<sup>1,2</sup>



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### Abstract

**BACKGROUND:** The prognostic value of tumor-infiltrating lymphocytes (TILs) in head and neck squamous cell carcinoma (HNSCC) remains controversial. Additionally, there is no standardized approach or cutoff value for evaluating TIL levels. The aim of this study was to establish a feasible method and criterion to assess TIL levels for future clinical practice and research use and to explore the relationship between TIL levels and prognosis. **PATIENTS AND METHODS:** This retrospective cohort study reviewed the records and pathological sections of 202 patients with HNSCC who were surgically treated at Beijing Stomatological Hospital, Capital Medical University, from January 1998 to January 2011. The predictor variable was the TIL level. The main outcome assessment parameters were disease-free survival (DFS) and disease-specific survival (DSS). **RESULT:** The T stage ( $P = .008$ ), smoking history ( $P = .042$ ), alcohol history ( $P = .048$ ), need for radiotherapy ( $P = .012$ ) and microscopic extracapsular spread (ECS) ( $P = .012$ ) were associated with the TIL level. A cutoff value equal to 70% could be taken as a threshold for TIL assessment, with a TIL level higher than 70% associated with a better prognosis (DFS rate: 51.9%,  $P = .018$ ; DSS rate: 59.3%,  $P = .049$ ). The Cox regression model showed that the TIL level was an independent prognostic factor for DFS (hazard ratio (HR): 0.786, 95% CI: 0.618-0.999,  $P = .049$ ). **CONCLUSION:** The TIL level is closely related to the prognosis of patients with HNSCC. A threshold value of 70% is appropriate for TIL assessment, as patients with a TIL level higher than 70% show a better prognosis. Thus, the TIL level might serve as an independent predictor for HNSCC recurrence.

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### Introduction

Approximately 635,000 new cases of head and neck cancer are diagnosed each year worldwide, and over 90% of cases are squamous cell carcinoma [1]. The treatments for head and neck squamous cell carcinoma (HNSCC) are improving; however, despite surgery with or without combined chemo-radiotherapy, the 5-year survival rate and the recurrence rate remain poor [2]. Thus, novel variables that can provide valuable information about prognosis are urgently needed for HNSCC.

Tumor-infiltrating lymphocytes (TILs) have been used to predict the prognosis of solid tumors for many years, as these cells serve as a readout of the interaction between the immune system and cancer cells [3]. There is emerging evidence to support the positive role of TILs in HNSCC, but there remain some controversial issues, and no standard process has been established for the evaluation of TILs. The

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immunoediting theory distinguishes the relationship between tumor cells and immune cells based on three phases: elimination, equilibrium and escape. This relationship persists for the entire duration of cancer progression [4]. Based on this theory, the immune system can have a positive effect by eliminating tumor cells, although the immune microenvironment may promote malignant cells to become more aggressive and capable of escaping immunological surveillance [5,6]. Although current studies have presented arguments about the function of TILs, cumulative data suggest that these mononuclear cells are associated with favorable outcomes in squamous cell carcinoma and the response to chemo-radiotherapy [7,8].

To explore the relationship between TILs and malignant tumors, formal approaches to evaluate the level of TILs exist in many fields, with the exception of HNSCC, and the TIL level has been applied in the clinic and in research trials [9,10]. Some pathologists even believe that TILs possess more predictive value than conventional TNM staging.

In regards to HNSCC, the literature on TILs provides limited evidence of their prognostic role, and many findings have been based on small sample sizes [11]. Moreover, the different evaluation criteria used make the results of different studies incomparable. Due to these issues, an acceptable method to assess the level of TILs in HNSCC is urgently needed. Based on the methods used to evaluate TILs in other solid tumors (e.g., breast cancer, colorectal cancer, etc.) [9,10,12], in this study, we sought to establish a standardized procedure for measuring the TIL level and confirm the prognostic role of TIL levels in HNSCC.

In this study, we used hematoxylin and eosin (H&E) staining to observe and assess the infiltration of lymphocytes in the tumor stromal area and then analyze the relationship between TIL level and recurrence or survival rate. We focused on establishing a feasible approach and criterion to evaluate TILs, with the hope that the devised method can be used in common clinical practice and research for pathological diagnosis and prognostic adjudication.

## Methods

### Patient Samples

This research was conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki (2002 version) and with approval of the Institutional Review Board of the Beijing Stomatological Hospital of Capital Medical University. Patient inclusion criteria included the following: (1) patients with a pathological diagnosis of squamous cell carcinoma; (2) patients with a primary tumor without evidence of distant metastasis; (3) patients who were treated primarily with surgery; (4) patients with no previous treatment; (5) patients with complete clinicopathological data and available tissue specimens; and (6) patients with a tumor located in the tongue, lower gingiva, upper gingiva, buccal mucosa, floor of the mouth, oropharynx, or hard palate. The exclusion criteria included preoperative chemotherapy or radiotherapy, failure to undergo surgery and the inability to obtain pathological slices. The medical records of patients who met the inclusion criteria between January 1998 and January 2011 for HNSCC at the Department of Oral and Maxillofacial-Head and Neck Oncology, Beijing Stomatological Hospital, Capital Medical University were retrospectively retrieved. All of the slides from the primary tumor sites for the selected patients were obtained.

### Management

All patients underwent surgery for resection of the primary tumor. The surgical procedure was selected by the surgeon according to the tumor site and local practice. Standard surgery, including radical tumor resection, neck dissection and the reconstruction of tissue defects (as necessary), was performed. Local excision of the primary site was performed with a minimum margin of 15 mm. Patients who had UICC stages pT3 and 4, pN+, perineural invasion and/or vascular emboli were recommended to receive radiation treatment, whereas patients who presented with extracapsular spread and/or positive margins were recommended to receive chemo-radiotherapy.

All tissue samples were obtained during the surgery and stored in paraffin. Tissues embedded in paraffin were sectioned into 4  $\mu$ m sections and placed on a slide. After applying a standardized process for H&E staining, the slides were observed under a microscope (Olympus BX61) and scored using the approach described below. Samples from patients with oropharyngeal squamous cell carcinoma were also stained to assess the p16 status.

### Approach and Threshold

For this study, we analyzed a significant amount of literature on TILs, and the approach and criterion for evaluating the TIL level in the stromal area were as follows (Figure 1):

- a. Determine tumor area: We scanned the slide at low magnification and determined the boundaries of the tumor area. Only TILs inside the tumor boundaries were calculated.
- b. Remove necrotic and degenerated tissue: After sketching the boundaries, we removed necrotic and degenerated tissue inside the tumor area.
- c. Determine the type of tumor stromal area: The tumor stromal area can be divided into three categories: cell-rich, moderate and stroma-rich types. The cell-rich type is defined as more than 70% of the tumor area composed of carcinoma cells or tumor nest; when the stromal area is greater than 70%, it is classified as the stroma-rich type. The moderate type is defined as the intermediate situation.
- d. Assess TILs by quantifying only mononuclear cells (e.g., lymphocytes and plasmacytes); neutrophils in necrotic areas, dendritic cells and macrophages should be ruled out.
- e. Assess the percentage of TILs in the stromal area: We selected five typical views in each slide and assessed TILs score as the total stroma area divided by the lymphocyte-occupied area. The average value was considered the final score of the slide.

The thresholds for evaluation of the TIL levels were established as follows: (1) 0% < low level  $\leq$  30%; (2) 30% < moderate level  $\leq$  70%; and (3) 70% < high level  $\leq$  99%.

The slides were first observed under a magnification of  $\times 200$  (ocular  $\times 10$ , objective  $\times 20$ ). Then, the typical regions were used to represent the common situation inside the tumor and were evaluated under a magnification of  $\times 400$  (ocular  $\times 10$ , objective  $\times 40$ ). Each slide was evaluated by two pathologists, and the results were considered only when the difference between observers was less than 10%. When the difference was more than 10% or divided into different TIL subgroups, the two pathologists determined the final score by consensus.

### Statistical Analysis

The descriptive statistics used were frequencies, percentages, and means  $\pm$  standard deviations. The outcome assessment parameters

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