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Overall assessment of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma: Time to take notice

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Abstract

Background: The immune response has an important role in cancer progression. At the same time, anti-tumor effect of the immune response has been speculated to cause destruction of cancer cells. Recent studies have highlighted the significance of tumor-infiltrating lymphocytes (TILs) in different cancers. The International Immuno-Oncology Biomarkers Working Group has in 2017 published a practical review proposing a standardized method to assess TILs in the routine hematoxylin and eosin (HE) stained sections.

Aims/Objectives: To discuss the association between TILs and survival in head and neck squamous cell carcinoma (HNSCC).

Material and Methods: We review recent studies having assessed TILs in HNSCC and showing that tumors having low TILs associate significantly with worse survival.

Results: These findings underline that HNSCC cases with low TILs might benefit from multimodality treatment and might be indicated to receive immunotherapy.

Conclusions: The evaluation of TILs using routine HE-stained sections is practical and can be considered in assessment of antitumor activity.

Significance: Here, we highlight the impact of overall assessment of TILs using the routine HE-stained specimens in HNSCC.

Keywords: Tumor-infiltrating lymphocytes (TILs); Head and neck squamous cell carcinoma (HNSCC); Clinical behavior.

Tumor-infiltrating lymphocytes

Immune response is a critical factor for cancer progression, and tumor evasion from immune response has been recognized as one of the hallmarks of cancer [1]. Immune signature, especially infiltrates of lymphocytes, in tumor samples has been associated with behavior of head and neck cancer [2]. Evaluation of immune response to cancer is an emerging prognostic indicator in various tumors [3]. To attack cancer tissues, immune cells move from blood to the tumor and are therefore known as tumor-infiltrating lymphocytes (TILs). In general, high infiltration of TILs as assessed in routine hematoxylin and eosin (HE) stained slides is associated with improved survival of different solid tumors [4-7].

In head and neck squamous cell carcinoma (HNSCC) TILs have been widely studied and have shown high impact [8-15]. For example, our group recently reported the significance of TILs in early oral tongue squamous cell carcinoma (OTSCC) using HE-stained specimens [10]. The multivariable analysis of our multi-institutional study clearly showed that low expression of stromal TILs at the invasive front is associated with a worse clinical behavior in early OTSCC [10]. In human papillomavirus-positive (HPV+) oropharyngeal squamous cell carcinoma (OPSCC) the significance of TILs in predicting survival was reported by Ward et al. [8]. Similarly, Faraji et al. [11] recently reported a promising prognostic impact of TILs evaluated in HE-stained sections of early stage HPV+ OPSCC. This finding was confirmed in another recent study [9]. Our study [10] and the study of Faraji et al. [11] both found a significant association between the assessment of TILs and early-stage of HNSCC in two different subsites. Interestingly, in a cohort that included both oral and oropharyngeal SCC, Xu et al. [12] reported that the level of TILs is an independent prognostic indicator for disease-free survival and disease-specific survival. These studies refer to the eligibility of TIL scores assessed in HE-stained sections as a universal prognostic indicator for both HPV+ and HPV- HNSCC.

Furthermore, a recent large-scale cohort study on nasopharyngeal carcinoma (NPC) from China (i.e. endemic NPC) showed that the assessment of TILs forms a promising prognostic tool for survival and metastasis [14]. Furthermore, our multicenter study including a large nationwide cohort of NPC in Finland (i.e. non-endemic NPC) confirmed the prognostic significance of TILs assessed in HE-stained specimens [15]. In laryngeal squamous cell carcinoma, Wang et al. [13] reported that the density of TILs assessed in HE-slides can independently predict recurrence and overall survival. All the above-mentioned studies have shown that the assessment of TILs using HE-stained sections is simple and has a promising prognostic value in different subsites of HNSCC.

Clinical significance of TILs in era of immunotherapy for HNSCC

Harnessing the immune response for cancer treatment, i.e. immunotherapy, has been tested for many cancer types. Patients with recurrent or metastatic HNSCC who do not respond to conventional chemotherapy protocols might benefit from immunotherapy. Of note, nivolumab which is a monoclonal antibody against anti-programmed death 1 (PD-1) was the first immunotherapy for recurrent HNSCC (that was refractory to platinum-based therapy) and was approved in 2016 by the US Food and Drug Administration. Such approval emphasizes the critical importance of the immune system in pathogenesis of HNSCC [16]. Early identification of those HNSCC cases that will need augmentation of the antitumour immune response can improve their survival. Several recent studies have reported a strong association between TILs and the behavior of HNSCC [8-15], and thus TILs having potential as tools to identify HNSCC cases that might benefit from immunotherapy. Such identification of aggressive HNSCCs can be initiated by overall assessment of TILs using routine HE-stained sections. Then further immunostaining

analysis for cases with low TILs can include specific immune checkpoints (e.g. PD-1 and its ligands, PD-L1). Of note, Topalian et al. have reported a significant relationship between the expression of PD-L1 in tumor cells and the response to immunotherapy in cancers of different histologic types although their cohort did not include HNSCC [17]. Therefore, it is necessary to examine the usefulness of assessment of TILs (overall and specific immune biomarkers) as a tool for predicting response to immunotherapy approaches that are based on immune checkpoint inhibitors in HNSCC.

Overall visual assessment of TILs

Recent guidelines introduced by the International Immuno-Oncology Biomarkers Working Group in 2017 have emerged as a simple and cost-effective method for the assessment of TILs in different cancers [4, 10, 15]. Due to its high inter-observer agreement, this method can be proposed as a standardized criterion for the assessment of TILs. Moreover, it has been shown to be a promising prognostic tool in studies of different HNSCC sites with good reproducibility [9, 10, 15]. The proposed method applies diagnostic HE-stained cancer sections for the evaluation of TILs as a continuous parameter in percentage (5%, 10%, 20%, 30%... etc).

The recommendations for overall assessment of TILs using HE-stained sections have been discussed in recent publications [3, 18]. In brief, assessment of mononuclear immune cells should be considered in two categories: stromal and intra-tumoral (intra-epithelial) categories. Stromal TILs refer to the percentage of stromal area occupied by lymphocytes. Intra-tumoral TILs refer to the percentage of tumor islands occupied by infiltrating lymphocytes. Usually the stromal and intra-tumoral TILs are correlated [10, 15]. Stromal TILs should be assessed in the stroma that is related to the tumor. It is recommended to consider the average of TILs and not to focus on

hotspots. Areas of necrosis should be excluded. In addition, polymorphonuclear leukocytes should be excluded from the scoring. One full cancer section (HE-stained) that best represents the tumor can be enough. However, assessing tumor tissue microarray samples might not be enough to obtain an average value and thus, it might lead to focusing on hot spots, which is not recommended.

In HNSCC studies that assessed TILs using HE-stained sections a low density of TILs associated with worse survival [8-15]. However, the cutoff point to stratify the tumors into low TILs or high TILs is not yet established for each subsite of HNSCC. In our recent analysis of nasopharyngeal cancer, we reported that 5% was the most optimal cutoff to identify the cases at high risk. In oropharyngeal cancer, two studies have used 20% as the cutoff point to distinguish tumors with low TILs [8, 9]. The same cutoff of 20% has been found as the most optimal also for early oral tongue cancer [10]. However, this issue still requires validating studies to re-examine the currently proposed cutoff points and to obtain a more definitive conclusion on the most optimal cutoff point for each subsite of HNSCC. In addition, the published studies have assessed TILs in postoperative cancer specimens, and further research should compare the scores of TILs in incisional biopsies and postoperative samples.

Image analysis of TILs

Recent research has widely used artificial intelligence and image analysis techniques for the assessment of prognostic markers for survival prediction with special emphasis on routine HE-stained sections [19]. Of note, a recent study on oral squamous cell carcinoma has described a novel digital score for assessment of TILs on HE-stained slides [20]. Such a digital method can aid in fast, precise and standardized assessment of TILs. However, there is still a lack of studies investigating this in various subsites of HNSCC. Therefore, future research on TILs in HNSCC

should consider applying machine learning techniques. Indeed, large cohort/s for the purpose of effective training of neural networks are needed in machine learning, and this warrants a multi-institutional collaboration.

Outlook

Rapidly advancing research on cancer immunotherapy and its clinical usefulness in HNSCC management have opened new avenues for research on biomarkers for the identification of patients who will benefit from immune therapies, and for the assessment of responses to such treatment. Emerging evidence indicates that overall assessment of TILs can be a promising prognostic tool in HNSCC and other cancers. Further research should consider using digital pathology-based quantification and artificial neural networks to analyze the impact of TILs in different subsites of HNSCC. Development of a web-based tool for assessment of TILs can be a step forward towards standardization of assessment of TILs. Based on currently available data, it seems possible and of great importance to include assessment of TILs in the routine pathology report for HNSCC using routine HE-stained tumor sections.

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