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Utility of CD8 score by automated quantitative image analysis in head and neck squamous cell carcinoma

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ABSTRACT

Introduction: In head and neck squamous cell carcinoma (HNSCC) high numbers of tumor infiltrating CD8 T cells in the tumor microenvironment are associated with better outcome. However, no investigators have employed automated image analysis on whole slide images to permit CD8 scores for use in clinical practice. The aim of this study was to develop and validate an image analysis algorithm to automatically quantify CD8 T cells in patients with oropharyngeal HNSCC.

Materials and Methods: Using brightfield image analysis results were cross-validated with fluorescence based quantification (AQUA™). A nuclear image algorithm designed to run on whole slide images was optimized to manual count. The algorithm was locked down and used on a cohort of whole tissue sections from HNSCC patients. Multivariate clinicopathologic parameters and outcomes were statistically correlated with image analysis results.

Results: Linear correlation between manual counts and the customized CD8 algorithm was 0.943. A total of 74 oropharyngeal HNSCC cases were analyzed for CD8 immune cell infiltrate using this image analysis algorithm. A CD8 immune cell density above 136 cells/mm^2 was associated with median survival of 18 years compared to 5 years. When multivariate modeling was performed, HPV infection was the only predictor of survival; however, when HPV was excluded only CD8 cell density predicts survival.

Conclusions: We report the successful technical development and clinical validation of an image algorithm to automate CD8 immune cell density for oropharyngeal HNSCC. Employing brightfield image analysis on entire tumor sections instead of tumor subcompartments permits this strategy to be widely implemented.

Introduction

Tumor infiltrating lymphocytes (TILs) have become an important measurement in many organ sites as their presence correlates with prognosis and for some tumors TILs also have therapeutic implications [1]. Among these TILs, CD8 T cells have been shown to be tightly associated with cancer patient survival and the predominant effector for cancer immunotherapy [2,3]. Cytotoxic CD8 T cells are able to directly kill neoplastic cells through direct lysis. Accordingly, many immunotherapy approaches have sought to target and enhance tumor-reactive CD8 T cells [3,4].

For head and neck squamous cell carcinoma (HNSCC) high numbers of tumor infiltrating CD8 T cells in the tumor microenvironment (TME) are associated with oropharyngeal localization, limited tumor growth and a lower incidence of smoking [5,6]. Patients with HNSCC that exhibit high infiltration of CD8 T cells also demonstrate a significantly better outcome [5,7–16]. In HNSCC human papillomavirus (HPV) infection is associated with better prognosis, which appears in part to be due to enhanced immune activation in the TME, and especially enhanced infiltration of CD8 T cells [16–18]. Given the direct relationship between TILs and HNSCC, a direct method to assess an immune response within HNSCC would be desirable [19].

The assessment of the CD8 T cells in patients afflicted with HNSCC has been attempted using flow cytometry either on procured tissue biopsies or from peripheral blood [8,20]. Attaining such a “CD8 score” may be of value to help determine prognosis and treatment before definitive surgical resection. An additional benefit of employing flow cytometry for this purpose is the multidimensional interrogation of immune cells that can be performed by this method. However, there are conflicting studies about whether systemic CD8 T cell levels correlate

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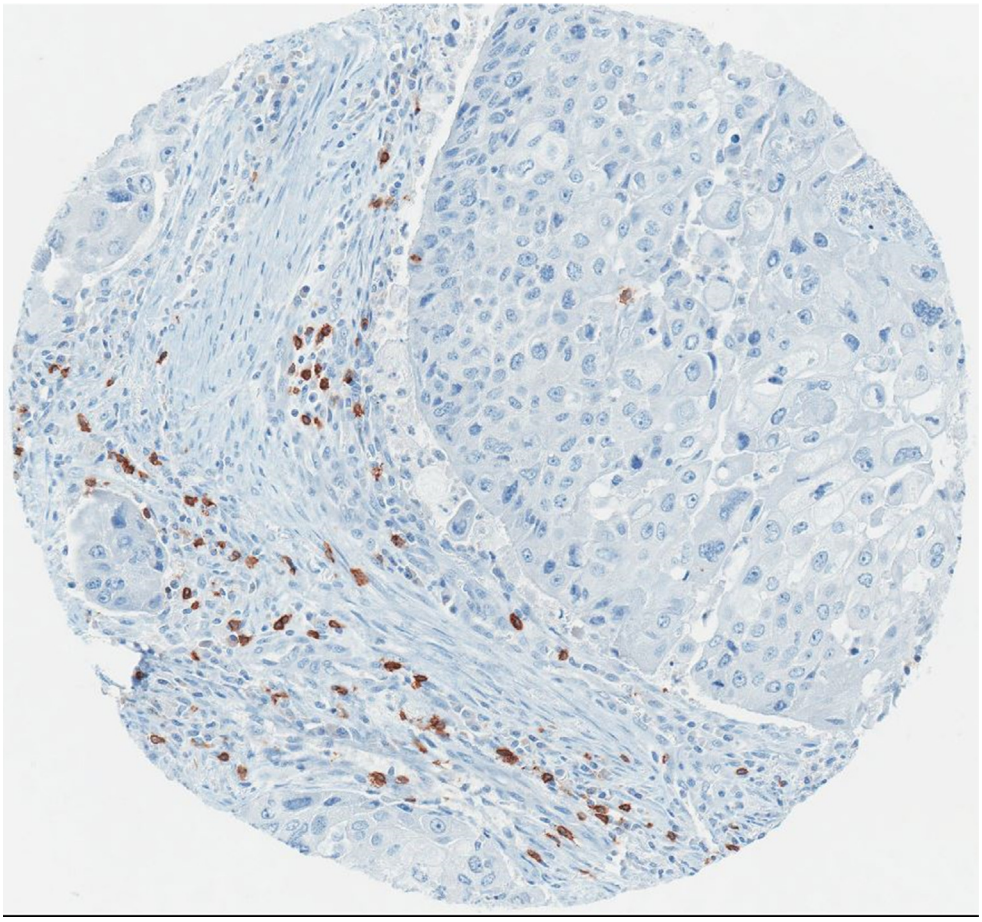

Fig. 1a. Example of a core from the YTMA stained by CD8 immunostain.

with tissue CD8 T cell infiltration.

Since there are no distinct morphologic features of CD8 T lymphocytes present in hematoxylin and eosin (H&E) stained tissues, an immunohistochemical (IHC) stain for CD8 T cells is necessary. The assessment of CD8 T cells within immunostained tissue sections has generally been reported in quantitative and semi-quantitative fashions. No specific method has yet been recommended to assess CD8 T cells and thus each published study assessing TILs in HNSCC has analyzed CD8 infiltrates using slightly different methods [19]. One method frequently exploited included counting the number of CD8 T cells in various high power fields and then averaging those values across these fields [7,9–11,13]. Many studies relied on manual counts from acquired still images or examining stained samples directly with a conventional light microscope. One group of authors utilized software (Count from Biomas) to perform cell counting [11,21]. The output of prior quantification studies in TILs in HNSCC has typically been reported as an absolute count of T cells or percentage/ratio to other immune cells. Few studies have provided quantification in the form of density (i.e. number of CD8 T cells per area of measurement) [8,22]. Using digital imaging, image analysis can be leveraged to perform this quantification [23].

Additionally, it is hard to extrapolate findings from different publications as these studies often quantified cells in different regions of tissue sections. The three main areas generally scored include the intratumoral/intraepithelial/tumor-host, peritumoral/tumor-stroma, and peripheral tumor compartments [5–7,12]. Specific descriptions of these tissue areas are lacking in these studies. Nevertheless, most investigations focused predominantly on the intratumoral component. Moreover, some of these studies were based on tissue microarrays while others used whole tissue sections. To the best of our knowledge, no investigators have employed automated image analysis of whole slide images to determine CD8 scores in oropharyngeal HNSCC.

The aim of this study was to develop and validate an image analysis algorithm to automatically quantify CD8 T cells in whole slide images acquired from immunohistochemically stained tissue sections from patients with HNSCC.

Methods

This study was approved by the University of Pittsburgh's Institutional review Board (IRB PRO 15070630) and Yale Human Investigation Committee protocol #9505008219 from Yale University.

The immunohistochemistry clone for CD8 was C8/144B (DAKO, Carpenteria, CA). Pre-treatment of tissue was performed and antibody was diluted to a concentration of 1:40. DAB (3,3′-diaminobenzidine) was used as the color reagent for visualization.

The best approach to validate a new assay is to compare it to a previously proven assay, and to compare both assays to outcome. To verify that the algorithm results were accurate, the findings of bright-field analyses were compared to equivalent assessments obtained using a fluorescent-based AQUA^{TM} system (NavigateBP, Carlsbad, CA) and to outcome in lung cancer [24]. For the standardization aspect of the study, sections from a Yale tissue microarray (YTMA) lung cancer cohort of 223 cases was stained with CD8 by immunohistochemistry at the University of Pittsburgh Medical Center (UPMC, Fig. 1a). The YTMA was constructed as previously described [24]. The YTMA of non-small cell lung carcinoma cases had 177 intact cores for evaluation. TMA slides were digitized using an Aperio AT2 scanner at 40x magnification. Once uploaded in eSlideManager (eSM) the digital slide was de-arrayed. Some adjustment was needed for the de-array circles in order to ensure the sections from the cores were completely contained within