Review Article

Besides and Beyond Histopathology; for Adjuvant Treatment in Early Tongue Cancer

Abstract

Oral tongue squamous cell carcinomas differ significantly from that of other subsites of oral cavity in relation to clinical behavior. They are more aggressive and have a poorer prognosis. The treatment of choice of early (stage I and II) tongue cancers is surgery. The need for adjuvant treatment is decided on the basis of the histopathology report of the surgical specimen. High-risk patients (positive surgical margins, perineural invasion, lymphovascular spread, lymph node metastasis, and extracapsular extension) receive adjuvant treatment while others are observed. Unfortunately, in the apparently low-risk patients who are observed, there is a high rate of locoregional failure. There are certain histopathology parameters though not routinely validated may be of prognostic significance in this subset of patients. In this review, we have highlighted the importance of the routinely validated and the nonvalidated histopathology parameters and their proper assessment in the decision-making for adjuvant treatment of patients with early tongue cancers.

Keywords: *Adjuvant therapy in tongue cancers, early squamous cell carcinoma tongue, indications for radiotherapy in tongue cancers, molecular markers in tongue cancers*

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Introduction

Oral tongue cancers are distinct epidemiologically and biologically from cancers of other subsites of the oral cavity. They are more common in females, patients aged below 40 years, and nonsmokers.[1-4] Rusthoven retrospectively compared survival in patients with early squamous cell carcinoma (SCC) of the oral tongue (cT1-2 N0 M0) with that in patients with SCC in other oral cavity subsites using the surveillance, epidemiology, and end results' database.[5] Six thousands seven hundred and ninety-one patients were identified of whom 40% had oral tongue cancers and 60% had cancers of other subsites of the oral cavity. The 5-year overall survival (OS) and cause-specific survival (CSS) rates were 60.9% and 83.5%, respectively, for patients with oral tongue SCC versus 64.7% and 94.1%, respectively, for patients with SCC of other oral cavity subsites ($P < 0.0001$ for both OS and CSS). He concluded that the prognosis of oral tongue cancer varies considerably as compared to cancer of other subsites of the oral cavity.

Treatment of stage I and II oral tongue cancers is primarily surgery. Surgery usually comprises of wide excision of the lesion with level I-IV selective neck dissection. In the busy oncological clinics, the decision for adjuvant therapy is based on certain fixed postoperative histopathological parameters. Patients with poorly differentiated tumors, close or positive margins, perineural invasion (PNI), lymphovascular spread, deep infiltrative tumors or nodal metastasis with or without extracapsular extension receive adjuvant therapy. The rest without any of these adverse features are kept under observation. However, in our clinical practice, a significant number of the "apparently low-risk patients" develop early locoregional recurrence and their prognosis is dismal. We searched the literature and found similar results in different retrospective case series [Table 1].[6-10]

This scenario raises a few pertinent questions.

Are we missing something in these patients?

Would adjuvant therapy have benefitted them?

Can histopathology report give us an answer?

Routine histopathology comprises assessment of certain parameters such as

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Table 1: Survival outcomes in early tongue squamous cell carcinoma - review of literature

Author	Year of publication	Study design	Results
Han *et al*.[6]	2007	Retrospective study (*n*=125)	5 years OS 62.59%
An *et al*.[7]	2008	Retrospective study (*n*=63)	5 years OS rate 97.1% in Stage I and 76.2% in stage II, and 5-years disease-free survival rate 76.7% in stage I and 43.5% in stage II
Sopka *et al*.[8]	2013	Retrospective study (*n*=126)	3- and 5-year actuarial local control 77% and 73%, respectively
Mantsopoulos *et al*.[9]	2014	Retrospective study (*n*=263)	The 5-year OS 56.9%, disease-specific survival rate 75.2% and local control was 86.3%
Yanamoto *et al*.[10]	2013	Retrospective study (*n*=58)	The 5-year disease specific and recurrence free survival 89.5% and 73.3%, respectively

OS – Overall survival

margins, tumor thickness, grade of differentiation, PNI, vascular invasion, and nodal status. However, beyond the usual known factors, quite a few histopathological factors come into our minds that are not routinely validated. Some of these are cost-effective and simple while others are costly and time-consuming.

This article is a review of the histopathological parameters (both validated and nonvalidated) that are of importance in deciding upon adjuvant therapy.

Validated Parameters

Assessed routinely, these factors are of prime importance in postoperative adjuvant therapy decision-making. However, the correct pathological interpretation of these at times can be tricky owing to the complex anatomy of the surgical specimen.

Margins

Time and again, the role of surgical margin has been emphasized in literature. Local tumor control is best achieved by complete surgical excision with "adequate" resection margins. Although the concept of positive margin is fairly straightforward (surgical "cut through" the tumor with tumor cells seen at the resected margins), considerable confusion surrounds the definition of "close" margins. Most studies that specifically define margin distance use a definition of ≥5 mm to define margin adequacy. Chen *et al*.[11] reported on 270 consecutive operated patients of oral cavity, oropharynx, hypopharynx, and larynx, using a defined 5 mm margin standard. Locoregional recurrence and 5-year disease-free survival (DFS) rates were 55% and 7% versus 17% and 39%, for patients with inadequate versus adequate margins, respectively. Similarly, Loree and Strong[12] reported the outcome for 398 consecutive patients with oral cancer, using a defined 5 mm standard for margins. Locoregional recurrence and 5-year OS rates were 30% and 52% versus 18% and 60% for patients with inadequate versus adequate margins, respectively.

Contrary to the mucosal margins which are visible, assessment of depth of resection requires intraoperative palpation of the specimen. Woolgar *et al*.[13] in a review of 301 patients with oral and oropharyngeal cancers operated with curative intent found 87% of the inadequate margins (61/70) were at the depth as opposed to only 16% at the mucosal margins (11/70). Less than 2% of 301 resections had inadequate margins solely on the basis of mucosal margins.

How to measure the surgical margin?

The resected surgical specimen should initially be grossly examined by the surgeon to assess for the mucosal and the deep margins. This includes apart from visual examination, thorough palpation of the specimen to identify any induration along or close to the resected margins. This is especially essential at the depth where the tumor is usually infiltrative. The closest gross margin should be marked and sent to the pathologist for assessment. Sections should be taken from the tumor invasive front to the nearest surgical resection edge in a "perpendicular direction" and measured in millimeters. This is in distinction to parallel, *en face* margins, which assess greater surface area, but do not allow for the measurement of margin distance.

The treatment of patients with positive or close resection margin is reexcision or adjuvant therapy. Reresection is technically difficult in tongue cancers as opposed to bone revision due to two reasons. First, the exact site of close margin is difficult to assess due lack of definable landmarks. Second, tongue being a muscular structure, the remnant part retracts deep in the musculature after initial resection. Hence, in most cases, inadequate margins would need to be supplemented with adjuvant treatment, based on the comparative analysis of two randomized clinical trials.[14]

Most guidelines including the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology guidelines advocate adjuvant chemoradiotherapy for positive margins and not for close margins.[15,16] We also advocate similar treatment protocol. However, a close margin should be analyzed meticulously for any foci of margin positivity before subjecting the patient to less aggressive adjuvant treatment (radiotherapy [RT] only).

Tumor Thickness

Tumor thickness denotes the maximum perpendicular dimension of the tumor measured from the surface

of the lesion to the deepest point, whereas depth of invasion represents the extension of the tumor beneath the epithelial surface. Tumor thickness has been shown as an independent risk factor for locoregional recurrence in many studies. In a cohort of 85 patients with oral tongue carcinoma, Yuen *et al.*[17] found tumor thickness to be a significant predictor for nodal metastasis, local recurrence, and DFS. In multivariate analysis, tumor thickness was the only predictor for nodal metastasis. Woolgar[18] demonstrated that the mean reconstructed thickness for tumors with pathologically positive nodes was 19 mm as compared to 10 mm for pathologically negative nodes. Fukano *et al.*[19] showed in 34 patients that the incidence of cervical metastasis increased from 5.9% for tongue carcinomas <5 mm thick to 64.7% for tongue carcinomas >5 mm thick. Brown *et al.*[20] noted that 38% of patients with tumor thickness <3 mm developed regional disease, compared with 41% of patients with tumor thickness of 3 mm to 7 mm and with 55% of patients with tumor thickness >7 mm. He also showed that increasing tumor thickness is associated with greater PNI. Fakih *et al.*[21] noted that in T1 and T2 SCC of the oral tongue, a thickness >4 mm is associated with a greater risk of neck relapse. In another recent study from our own institution, Thiagarajan S *et al.*[22] found a tumor thickness cutoff of 11 mm significantly affected the OS. In a retrospective analysis of 164 patients of stage I and II oral tongue SCC who underwent partial glossectomy with ipsilateral neck dissection without adjuvant RT, Ganly *et al.*[23] found regional recurrence rate was 5.7% for tumors <4 mm and 24% for tumors ≥4 mm thick. Multivariate analysis indicated that tumor thickness was the only independent predictor of neck failure ($P = 0.02$).

Traditional tumor-nodes-metastases (TNM) staging does not incorporate the third dimension, i.e., the tumor thickness. Thus, pTNM may understage the disease, especially in infiltrative tumors.

Although thickness is an independent risk factor for regional recurrence, there has been no consensus on the benefit of adjuvant RT in management of early oral tongue cancer with increased thickness. No prospective randomized trial has ever been conducted addressing this issue.

Our institutional practice is to radiate tumors which are ≥1 cm in thickness even in the absence of other adverse prognostic factors.

Perineural Invasion

PNI is defined as tumor invasion of the perineural sheath or epineurium. A study by Brown *et al.*[20] demonstrated that the presence of PNI decreased the 2-year survival from 82% to 52%. Lydiatt *et al.*[24] in a study of 156 patients with stage I and II tongue cancer found that local control rate at 5 years was 38% in patients with PNI versus 78% in patients without PNI. Thiagarajan S *et al.*[22] found PNI

to significantly affect DFS. Thus, extensive PNI is a clear indication of adjuvant RT.

Despite the clear importance of PNI, the percentage of mucosal SCC positive for PNI varies in literature from 5%[24] to 52%.[25] This discrepancy results from identifying PNI only in large diameter nerves. However, the presence of PNI in small unnamed nerves may not be clinically apparent; but the association between PNI and prognosis is independent of the nerve diameter.[25] Thus, the pathologist must look for PNI along unnamed nerves also while microscopically examining the histopathological specimen.

Confusion arises in patients with focal PNI without any other adverse tumor factor, whether to give adjuvant RT or not. Although there are no clear cut guidelines, the NCCN treatment guidelines for head and neck cancer considers PNI as an adverse factor and most oncologists recommend adjuvant RT in patients having PNI. In a recent study assessing the role of adjuvant radiotherapy in early tongue cancers with minor risk factors (MAFS), PNI was found to have a significant impact on disease free survival (DFS). Patients with MAFS receiving adjuvant RT had improved DFS as compared to those undergoing surgery alone.[26]

Vascular Invasion

Vascular invasion is defined as the presence of neoplastic epithelium in the endothelial lined vascular channels. Larsen *et al.*[27] in a study of 144 patients of head and neck carcinoma found vascular invasion to be present in >50% of pathological specimens. These patients had significantly more chances of harboring concomitant neck nodal metastasis and had increased incidence of distant metastasis. In another study by Close *et al.*,[28] the presence of vascular invasion corelated with increased risk of subsequent locoregional recurrence. Microscopically, vascular involvement is typically seen at the invasive front of the tumor and a perivascular lymphocytic infiltrate, including lymphoid aggregates, should raise the possibility of vessel involvement.

The presence of vascular invasion is an indication of adjuvant RT.

Histological Grading System

Although not routinely reported and not incorporated in the TNM staging system, histological grading system provides immense information in treatment planning. Initially proposed by Broders[29] and subsequently modified by Jakobsson *et al.*[30] and Anneroth *et al.*,[31] this grading system incorporates five histological parameters - degree of keratinization, nuclear polymorphism, number of mitosis (high-power field), pattern of tumor, and lymphoplasmacytic invasion. Bryne *et al.*[32] applied this grading system to the most anaplastic fields in the most invasive parts of the tumor and named it invasive cell grading system (ICG) [Table 2].[32]

Morphological features	1	2	3	4
Degree of Keratinization	Highly Keratinized (>50% of cells)	Moderately Keratinized (5-20% of cells)	minimal Keratinization (5-20% of cells)	No Keratinization (0-5%)
Nuclear polymorphism	Little nuclear polymorphism (> 75%mature cells)	Moderately abundant nuclear polymorphism (50-75% mature cells)	Abundant nuclear polymorphism (25-50% mature cells)	Extreme nuclear polymorphism (0-25% mature cells)
Number of mitosis (high power field)	0-1	2-3	4-5	>5
Patterns of invasion	Pushing, well delineated infiltrating borders	infiltrating, solid cords, bands or strands	Small groups or cords of infiltrating cells (n>15)	Marked and widespread cellular dissociation in small group of cells (n<15) and or in single cells
Host response (lympho-plasmacytic infiltrate)	Marked	Moderate	Slight	None

Table 2: The invasive cell grading system

Bryne *et al.* in two cohorts of 68 and 61 patients with oral cavity SCC showed that ICG was an independent and significant risk factor for survival. Patients with a score between 5 and 10 experienced a 57% survival as compared to 19% in patients with a score >10.[32,33]

Spiro *et al.*[34] retrospectively assessed the pattern of invasion in 150 patients of oral tongue and found that an endophytic growth pattern were associated with a significant increase in local recurrence ($P < 0.04$). With higher grades of infiltration (Grade 3 or 4), the tumors tended to be larger and the patients younger. Although the likelihood of nodal involvement and subsequent distant metastasis was significantly greater in those with Grade 3 or Grade 4 patterns ($P < 0.0003$ and < 0.01, respectively), there was no impact on local recurrence. Cumulative survival was significantly reduced when the pattern of tumor invasion was of higher grade ($P < 0.01$).

In addition to growth pattern, lymphoplasmacytic infiltration also has prognostic significance. It denotes the host immugenic response against the tumor. Anneroth *et al.* found that the presence of intra- and peri-tumoral infiltration decreased the chances of cervical lymph node metastasis.[32]

The commonly used NCCN guidelines do not include poor tumor differentiation as an adverse risk factor requiring adjuvant treatment.[15] Although there is no robust evidence, we prefer treating poorly differentiated SCC with adjuvant radiation even in the absence of other adverse factors.

Nonvalidated Parameters

These parameters widely range from simple cost-effective to costly and time-consuming ones. Not routinely reported, these can at times be of immense importance in planning adjuvant treatment.

DNA Ploidy

DNA nondiploid tumors behave more aggressively as compared to DNA diploid tumors. Byers *et al.*[35] in a series of ninety-one patients of oral tongue cancer found that DNA aneuploidy was an independent predictor for nodal metastasis. Hemmer *et al.*[36] in a series of 47 patients found DNA aneuploidy significantly increased tumor size and poorer grade of differentiation as compared to the DNA diploid tumors.

The role DNA diploidy needs to be evaluated in tongue cancers and this simple time and cost-effective parameter can easily be included in routine histopathology reports.

Ki 67 Index

Ki 67 is a nuclear protein and is a cellular marker of proliferation. Its role has been well validated in carcinomas of the prostrate, brain, breast, and nephroblastoma. In a study by Valente *et al.*,[37] Ki 67 immunostaining was used to predict the response to RT in oral SCC. Thirty-one cases of SCC were stained at diagnosis and after 10 Gy of RT. The percentage difference of Ki67 positive cells among the biopsy specimens taken at the beginning and after 10 Gy was correlated with the clinical response obtained at the end of the treatment and its significance determined. The percentage of Ki67 positive cells at diagnosis had no significant correlation with the final therapeutic outcome. By contrast, a decrease in the growth fraction after 10 Gy of RT was significantly correlated with the complete response ($P < 0.01$). Thus, the authors concluded that Ki 67 index can be a good prognostic marker after the first week of RT to separate the good versus poor outcome patients. A high Ki67 index denotes poor tumor biology and further research needs to focus on the role of adjuvant radiation in tumors with high Ki67 index.

Vascular Density

Vascular density is the determination of the number of microvessels in tissue and is of importance in cancer owing to angiogenesis and lymphatic spread. Tongue squamous carcinoma is notorious for lymphatic spread. Lymphatic vascular density (LVD) measurement is a marker of regional spread and thus aggressiveness. It is measured using immunohistochemistry. Yan *et al.*[38] compared the LVD in normal tongue and SCC tongue tissue and found that LVD was higher in malignant tongue tissue. OS was significantly shorter in patients with high LVD. The measurement of vascular density and its relation with RT is still at the preliminary level. Chen *et al.*[39] reported that single or fractioned doses of radiation decreases the vascular density in adenocarcinoma of mouse prostrate. However, routine reporting of this parameter may help us understand better the role of RT on vascular density and prognostic significance of the latter.

Molecular Markers

Transformation of a normal cell to a malignant cell is a result of multiple molecular events occurring at the level of protooncogenes and tumor suppressor genes. Each molecular event carries its own prognostic and therapeutic implications. These are being extensively studied and are the basis of novel targeted therapies.

p53, the guardian of the genome, is a vital constituent of the G1S checkpoint and an inducer of apoptosis in cells undergoing genotypic damage. Loss of this tumor suppressor gene, mostly by homozygous deletion, occurs in >60% of head and neck cancers. Hegde *et al.*[40] found that mutations in the p53 gene were associated with unfavorable overall and DFS in a group of 39 patients with head and neck cancer. Furthermore, the response to therapy was poorer in the mutated group of patients.

Atula *et al.*[41] studied p53 mutations in tongue cancer and found mutations in 54% of the samples. The mutations correlated with tumor size and grading. Other studies have demonstrated that p53 mutation precedes and favors the appearance of metastasis.[42,43] Thus, p53 mutation is associated with aggressive nature of tongue cancers and in general head and neck cancers.

Although human papilloma virus (HPV) 16 and 18 has emerged as one of the major carcinogens in head and neck squamous cell cancers, its role in oral tongue cancer seems somewhat overrated. HPV is a major carcinogen for base of tongue SCC. Most studies assessing HPV DNA or p16 assay for tongue lesions have not separately categorized oral tongue from base tongue lesions. Kantola *et al.*[44] found that none of 105 mobile tongue cancer patients harbored HPV. Two other studies by Dahlgren *et al.*[45] and Liang *et al.*[46] have reported HPV frequencies in oral tongue cancer of 2.3% and 1.96%, respectively, thus confirming its small etiopathogenetic role, at least in the mobile portion of the tongue cancers as compared to HPV-negative tumors.[45]

HPV positive tumors are prognostically better as compared to HPV-negative tumors.

Epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane glycoprotein located on chromosome 7p12. Its main ligands epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α), bind to the extracellular domain of EGFR and thus lead to the downstream activation of *ras* oncoprotein, which ultimately leads to cell cycle progression, decreased apoptosis, increased angiogenesis, and thus metastatic potential. EGFR and its ligand TGF-α are overexpressed in >90% of head and neck cancers. Data regarding the expression and prognostic value of EGFR in tongue cancer are limited. However, its overexpression is typically associated with greater radio- and chemoresistance and shorter DFS and OS. The development of monoclonal antibodies (MABs) against EGFR has been a milestone achievement in treating these cancers with otherwise dismal prognosis. Cetuximab, the prototype drug, has been shown to improve locoregional control, progression-free survival and OS when combined with RT as compared to radical RT in advanced head and neck cancers in a phase III randomized control trial.[46] Elderly patients who cannot tolerate chemotherapy and those with medical illness not fit for chemotherapy can be treated with this novel drug.

Vascular endothelial growth factor (VEGF) is a family of proteins with specific angiogenic potential. There are limited data concerning the role of VEGF overexpression and tongue cancer. Kim *et al.*[48] studied the expression of VEGF in 38 oral tongue cancer patients and found a significant correlation between VEGF expression and the extent of tumor invasion ($P = 0.002$). Furthermore, the tumor-free survival of the VEGF-positive patients was significantly worse than that of the VEGF-negative patients ($P = 0.019$).

Next Generation Sequencing

Head and neck cancers are predominantly an environmental disease caused by tobacco, alchohol, and HPV. However, many patients, especially of tongue cancers, are young without any habits. This group of patients' harbor-aggressive disease which is clinically and histopathologically a distinct entity highlighting the role of genetic factors in carcinogenesis.[49]

NGS accelerates the process of studying DNA by generating digital and quantifiable data that can be mapped back to the genome. Findings from next generation sequencing (NGS) studies of head neck squamous cancer will help us better understand the genetic aspects of a tumor traditionally considered environmental. This may open up a completely new avenue of approach and treatment based on targeted therapy.

Assessment of molecular markers and NSG are costly and time-consuming. Their role is limited in routine histopathological analysis. However, they can de used in

atypical scenarios such as young patients, no addiction, multiple primaries, and thus help develop targeted therapies based on the genetic mutation.

The role of molecular markers is further highlighted in treatment of patients with recurrent cancers where immunotherapy possibly holds a promising role. As compared to non selective cytotoxic chemotherapeutic agents, immunotherapy has more specific targeted action thus reducing the non desirable cytotoxic effects commonly occurring with conventional chemotherapeutic agents. Molecular marker analysis like EGFR, PD-1, PD-L1 can help choose patients suitable for a specific targeted therapy as well as monitor the treatment response.

The most commonly used targeted agents in cancer treatment are monoclonal antibodies (MAB). Cetuximab is the only MAB approved for treatment of head neck SCC. It blocks EGF signals by targeting EGF receptors on the tumor cells. Additionally it helps in immune surveillance by activating antibody dependant cytotoxic activity (ADCC) resulting in cancer cell death.[50] A randomized phase III study demonstrated a significant benefit in OS and progression free survival of adding cetuximab to conventional palliative chemotherapy in recurrent or metastatic head and neck SCC.[51]

A humanized IgG4 PD-1-blocking MAB, pembrolizumab has been granted FDA approval for the treatment of melanoma. Its role is now being evaluated in head and neck cancers in various clinical trials including two phase III trials- Keynote 040 and 048. PD-L1 blocking MABs durvalumab, atezolizumab and avelumab are under investigation for head and neck SCC in trial settings.

In recurrent and metastatic head and neck setting post surgery and CCRT the EXTREME trial showed benefit of addition of Cetuximab in healthy individuals, recent phase III CheckMate 141 trial has shown significant longer over all survival with Nivolumab, an anti–programmed death 1 (PD-1) monoclonal antibody.[52] Although it has been approved by the US FDA presently it has high cost of treatment which recurrent two weekly.

Conclusion

Through this review, we have tried to highlight the importance of histopathology in the management of patients with early tongue cancer. In our opinion, histopathology in addition to routine parameters should also include some simple and cost-effective factors whose importance in clinical practice is underrated due to the lack of available data. We strongly propose the need for a large prospective trial looking into the prognostic significance of various validated and nonvalidated histopathological factors in early tongue cancers. This can redefine the importance of the commonly used parameters and also bring to light the significance of certain other factors which till date have not received adequate attention. Based on the significance of each parameter, a normogram needs to be formulated which will help establish a uniform guideline for the role of adjuvant treatment in early tongue cancers. This may at least to some extent improve the survival of this common but deceptive and aggressive disease.

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Conflicts of interest

There are no conflicts of interest.

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