Prognostic and Predictive Markers for Oral Squamous Cell Carcinoma: The Importance of Clinical, Pathological and Molecular Markers

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INTRODUCTION

Survival rates for oral cancer and tumors of the oropharynx, caused by tobacco and alcohol, have not improved for decades except in major treatment centers. Treatment failures remain frequent despite improvements in clinical and imaging investigations and advances in surgical and radiotherapeutic techniques.

Most of the prognostic factors listed below have a direct impact on the management of the patient. The role of the infiltrative margins, HPV infection, ploidy status and cell proliferation indices are still being assessed but may have a significant role in determining treatment in the future. In some published studies that are retrospective in nature, small sample sizes, using more than one standard protocol for the management and use of univariate analyses have contributed to difficulties in interpretation of data from these trials.

DEMOGRAPHIC FACTORS

Several demographic factors are known to affect prognosis and survival. Young people (under the age of 45 years) are known to have better survival.[1] While the role of gender is not clear, single and divorced persons have poor survival rates. The socioeconomic status also plays a role. People who lived in deprived areas had a relative risk of 1.25 (95% confidence interval [CI]: 1.15-1.35) of dying from their cancer.[2] Tobacco consumption had a significant influence on the prognosis of oral cancer patients ($P = 0.046$),[3] and continuing to smoke after treatment contributes to poor survival. In a recent study in London, UK, smoking cessation and reduction in drinking alcohol and drinking cessation led to a significant reduction in mortality at 3 and 5 years ($P < 0.001$).[4]
TUMOR STAGE AND NODAL STATUS

Union for International Cancer Control TNM (UICC TNM) staging,[5] used in the treatment planning, allows comparison of data. Higher T stage correlates with poorer prognosis.[6] The TNM classification of oral squamous cell carcinoma provides a reliable basis for patient prognosis and therapeutic planning.

Size and multiplicity of lymph nodes are taken into account when assessing prognosis from cancer. The incidence of occult metastases to the neck can range from 15% to 60% depending on the different diagnostic procedures adapted. Clinical palpation, imaging, ultrasonography-guided fine-needle aspiration cytology and sentinel node biopsy techniques allow assessment of cases prior to surgery. Clinically, lymph nodes are assessed for location, number, size, shape, consistency and fixation. Nodes are considered to be malignant if their size is greater than 1 cm, and particularly if they are hard and fixed. After surgery, the analysis of the specimen allows pathological staging. The presence of nodal metastasis is the most important prognostic factor for oral cancers.[7,8] An approximately 50% reduction in 5-year survival rate is seen with the development of lymph node metastasis in patients with squamous cell carcinoma of the oral cavity. Contralateral neck metastasis may be associated with higher distant metastasis as spread of tumor across the midline confirms aggressive behavior.

Extracapsular spread (microscopic or macroscopic) is related mostly to prognosis.[9] These authors recommend that extracapsular (microscopic) spread should be incorporated into pathological staging systems. In particular, the capsular rupture has the most significant prognostic influence.

TUMOR THICKNESS

Tumor thickness, which is objectively measurable, might influence the prognosis of early oral cancer. In general, median tumor thickness varies between 1.5 and 8 mm for T1 and T2 cancers. Tumor thickness of greater than 4 mm imparts a worse prognosis.[10] In a recent study, tumor thickness was the only independent predictor of neck failure: Regional recurrence-free survival was 94% versus 72% (P = 0.02) for tumors <4 mm versus ≥4 mm, respectively.[11]

PATHOLOGICAL GRADE (DEGREE OF DIFFERENTIATION)

There is consistent evidence of the value of tumor grade in determining prognosis: Higher grades equate to a poorer prognosis.[6,12,13] Grading is based on the degree of resemblance of the invading carcinoma to the normal epithelium and its ability to form keratinizing islands, and follows the descriptions in the World Health Organization classification.[14] The most aggressive area (at × 100 magnification field) is graded as well, moderately or poorly differentiated. Most oral carcinomas are moderately differentiated. Tumor differentiation may well relate to the presence of up- or downregulated genes/proteins, but this has not been comprehensively tested. However, this pathological grading system is widely used and is listed in the current AJCC staging form. The system suffers from interexaminer variability and sampling errors. Prognostically useful Byrne’s malignancy grading system[15] has been used to prognosticate oral cancers.[16]

PATTERN OF INFILTRATION

An infiltrative margin, as opposed to a smooth pushing margin, has been shown to be an adverse prognostic feature in the tongue, the supraglottis and the floor of the mouth.[17] More cells at the invasive front are proliferating compared with the center, confirming that this part of the tumor is likely to be more informative in determining the prognosis.[18]

PERINEURAL INFILTRATION

Perineural invasion (PNI) of the small nerves is an important predictor of outcome of patients and is a sensitive indicator for regional recurrence and distant metastasis.[19] The 5-year disease-specific survival for patients with and without PNI was 56.6% and 94.6%, respectively (P < 0.0001).

EXCISION MARGINS

With respect to the tongue, either dysplasia or tumor at the margins may predict local recurrence.[20,21] From a surgical point of view, a margin >5 mm is clear, 1-5 mm is close and <1 mm is involved. Incomplete resection leading to an involved margin or the presence of dysplasia at the margin is associated with a significantly increased risk of local recurrence. For T1 and T2 oral cancers, histological completeness of excision margins therefore reduces the recurrence at the primary site, making other histopathologic variables somewhat irrelevant.[22] An unexpected close surgical margin has been claimed to represent a bioaggressive tumor. It appears that the use of frozen sections to assess margins has not been shown to be beneficial.
**HPV INFECTION**

Several studies have addressed the role of HPV in head and neck cancer. Data indicate that in oropharyngeal tumors, HPV status was associated with younger age, absence of traditional risk factors (such as smoking and alcohol consumption), high proliferation indices, high grade, basaloid subtype and an inverse association with p53 nuclear immunoreactivity. HPV positivity contributed to a favorable outcome attributable to an increased sensitivity toward radiotherapy. There is, however, insufficient evidence to modify treatment intensity in these patients based on HPV status.

**GENETIC MECHANISMS**

Numerous molecular studies have been undertaken to find pathways that are altered in oral squamous cell carcinomas at gene-expression and protein levels, with a special emphasis on their prognostic significance. These could be considered under several pathways, such as signaling pathways, markers associated with the cell cycle/apoptosis, cell adhesion, cell motility and invasion, angiogenesis, immortalization and inflammation. Many gene-expression profiles are altered and under- or overexpression is reported [Table 1], and some of these alterations are correlated with prognosis.

The highest number of studies was conducted to evaluate the prognostic significance of p53. Among the nine included studies in a meta-analysis, the hazard ratio for p53 was 1.48 (95% CI: 1.05-2.11), suggesting a survival advantage for negative p53 status. However, the authors concluded that the current evidence on p53 was inconclusive. In a recent study, phosphorylated (p) epithelial growth factor receptor (EGFR) expression was observed in ~40% of the cases and in the multivariable analysis, this contributed to cause-specific survival.

The combined evaluation of two or more phenotypic alterations might provide more prognostic information on oral carcinoma. This has been demonstrated by examining the coexpression of p53/p-glycoprotein, coexpression of combined cytoplasmic and membranous EGFR and p53, coexpression of c-erbB-2,3 and 4, p16/cyclin D1 amplification and RAR alfa/p21 expression. Alterations in the Rb pathway have been shown to be of higher significance in Indian cancers. The prognostic effect of p-mTOR on the overall survival of oral squamous cell carcinoma (OSCC) suggests that this marker may serve as a reliable biological marker to

| Table 1: Gene-expression changes and prognosis of oral squamous cell carcinoma |
|-------------------------------------------------|-------------------------------------------------|-----------------------------|-----------------------------|
| **Pathways affected**                           | **Genes studied**                               | **Changes in expression**   | **Prognostic effect**       |
| Signaling pathways                             | pEGFR                                           | Over                        | Poor prognosis              |
|                                                | c-erb-2,3,4                                     | Coexpression                | Decreased survival          |
| Cell cycle/apoptosis                           | TP53                                            | Over                        | Poor survival               |
|                                                | P16 (INK 4A)                                    | High expression             | Improved survival           |
|                                                | P14 (ARF)                                       | Expression                  | Improved survival           |
|                                                | P21 WAF1                                        | Expression                  | Poor prognosis              |
|                                                | Cyclin D1                                       | Over expression             | Poor survival               |
|                                                | P63                                             | Over                        | Poor survival               |
|                                                | Suvivin                                         | Expression                  | Poor prognosis              |
| Adhesion/mobility degradation                  | E-cadhehin                                      | Reduced                     | Local regional failure      |
|                                                | P-cadhehin                                      | Reduced                     | Local regional failure      |
|                                                | S100 A4                                         | Loss                        | Poor prognosis              |
|                                                | CD44                                            | Loss                        | Metastasis                  |
|                                                | MMPs 2 and 9                                    | Increased                   | Poor prognosis              |
|                                                | TIMPs 1 and 2                                   | Expression                  | Poor prognosis              |
|                                                | Ezrin                                           | Over                        | Shorter survival            |
|                                                | Maspin                                          | Loss                        | Improved survival           |
|                                                | Nm23H1                                          | Deregulation                | Poor outcome                |
| Angiogenesis                                   | VEGF                                            | Elevation                   | Increased severity          |
| Immortalization                                | Telomerase                                      | Activation                  | Invasive                    |
| Prostaglandin synthesis                        | COX2                                            | Increased expression        | Poor outcome                |
identify high-risk subgroups (with poor prognosis) and as a guide to therapy. Furthermore, the high expression of p-mTOR suggests that this protein may be a promising therapeutic target in OSCC.\[14\] Metastatic gene markers have not been investigated in detail in oral cancer.

The presence of allelic imbalance at 3p22-26, 3p14.3-12.1 and 9p21 are reliable predictors of outcome. Loss of heterozygocity (LOH) at each of these regions has an approximately 36-times increased risk of mortality relative to a case with retention of heterozygocity at these loci.\[13\] None of these markers or combinations has been incorporated into large prospective clinical trials. At present, the overall evidence is insufficient to alter clinical practice or to consider aggressive treatment for subsets of patients identified on the basis of the use of molecular markers alone.\[15\] These markers are not yet available in routine practice. In the future, with further advances, new therapeutic approaches may become available to provide individualized therapy to patients based on the molecular analysis of a primary tumor.

**CONCLUSIONS**

Based on the current evidence, the Royal College of Pathologists, London,\[16\] has categorized prognostic factors by their levels of evidence (A-D), A being the best evidence and D being the least. Level B evidence exists for: Tumor size that is the major contributor to stage, depth of invasion, patterns of tissue invasion by carcinoma, PNI predicting more aggressive cancers, presence of bone involvement and extracapsular spread. Level B/C evidence exists for histological grade of differentiation and margin status. The College considers these factors as “Core Data Items” that are supported by robust published evidence and are required for cancer staging and optimal patient management and to determine prognosis.

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