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# Oral squamous cell carcinoma: Review of prognostic and predictive factors

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Oral squamous cell carcinoma has a remarkable incidence worldwide and a fairly onerous prognosis, encouraging further research on factors that might modify disease outcome. In this review article, the authors approach the factors that may exert influence on the prognosis and eventually guide the selection of patients for more aggressive therapies. Published scientific data was collected, selected, and grouped into 3 main clusters: patient-, tumor-, and treatment-related factors. Well established aspects are discussed, but also those less common or with only supposed usefulness. Disease staging, extracapsular dissemination, resection margin free of disease, and tumor thickness are factors with high influence on the prognosis. There has been an increasing interest in the study of tumor molecular factors, and some have been strongly correlated with the outcome, showing promising pathways for the future development of more effective prognosis systems and anticancer therapies. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:67-76)

Squamous cell carcinomas (SCC, Fig. 1) encompass at least 90% of all oral malignancies.<sup>1,2</sup> Oral cancer holds the eighth position in the cancer incidence ranking worldwide, with epidemiologic variations between different geographic regions (it is the third most common malignancy in south-central Asia).<sup>3</sup> The World Health Organization expects a worldwide rising oral squamous cell carcinomas (OSCC) incidence in the next decades. In the US, OSCC represents 2%-4% of the annually diagnosed malignancies, being responsible for 8,000 deaths every year.<sup>4,5</sup> In the US, at the time of diagnosis, 36% of patients have localized disease, 43% regionally spread disease, and 9% present distant metastasis.<sup>4</sup> In some western European countries, such as Belgium, Denmark, Greece, Portugal, and Scotland, there has been an upward trend in the incidence of OSCC. Increasing mortality rates have been observed for at least 2 decades in Eastern Europe, where OSCC comprises a real public health issue.<sup>6</sup> OSCC implies quite significant mortality and morbidity rates,<sup>1,2,4,5</sup> and in spite of the vast amount of research and the advances accomplished in the field of oncology and surgery, the mortality rates remain unchanged. This motivates the search of factors with prognostic relevance in order to better tailor the

individual management of OSCC patients. The purpose of this article is to list and discuss some of these factors, focusing also on some of the most promising.

## METHODS

A web-based search for all types of articles published was initiated using MEDLINE/PubMed, with the key words "oral," "cancer," and "prognosis." The search was subsequently refined. The sites of specialized scientific journals in the areas of oral and maxillofacial surgery, oral medicine, and oncology were also used. In order to achieve a concise and informative text all authors were engaged in the selection of the information to be used, first on an individual basis and the final choice accomplished by group consensus. We have subsequently devised a wide-ranging selection of factors with potential influence on the outcome of this disease, whether well established ones or more recent with only conjectural usefulness. No additional statistical analysis has been conducted. Three clusters of issues were formulated: patient-, tumor-, and treatment-related factors.

## REVIEW OF THE LITERATURE

### Patient-related factors

*Sex and age.* Apparently, there are no prognostic differences between males and females,<sup>7-9</sup> although some authors have reported lower survival rates in females, attributed to delay in seeking medical care and lower acceptance of treatment.<sup>10</sup> The correlation of prognosis with age seems controversial, and some authors show no relationship between them,<sup>7-9,11</sup> whereas others demonstrate worse prognosis in older patients.<sup>10,12</sup>

*Tobacco and alcohol.* Although some results deny any association between survival and smoked tobacco or alcohol consumption,<sup>7</sup> most authors report higher mortality in smokers and alcohol drinkers.<sup>10,12,13</sup> Betel

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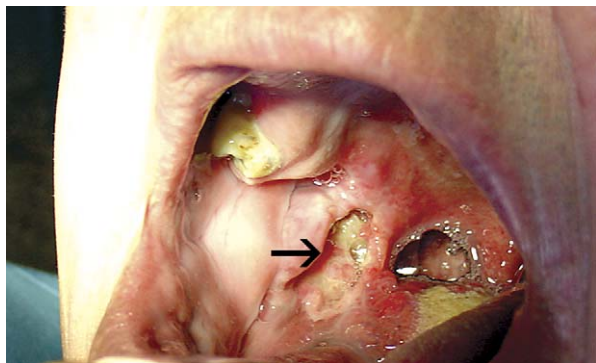


Fig. 1. Squamous cell carcinoma of the retromolar trigone, presenting as an ulcerated lesion (photograph taken with the patient's consent).

quid chewing (a common habit in some regions of Asia and some Asian communities in the western world) has been specifically correlated with poorer prognosis.<sup>7</sup> Smokers and alcohol drinkers seem to be at higher risk for the development of second primary oral cancer than nonsmokers and nondrinkers, thus facing more onerous outcomes.<sup>14-17</sup> This is also the case for those who maintain tobacco and alcohol consumption following diagnosis of the primary tumor.<sup>15-18</sup> Clinicians should therefore make every effort to persuade all patients (including those already treated for OSCC) to abandon these detrimental habits.

**Socioeconomic conditions.** Apparently, the outcome is somewhat worse for patients with lower socioeconomic status and education, most likely because of poorer oral hygiene and more difficult access to medical care.<sup>10</sup>

**Diagnostic delays.** It seems highly likely that diagnostic delays raise the probability of higher tumor growth and spread, consequently aggravating the prognosis. However, an extensive review concerning OSCC pointed out that the available data fail to demonstrate this thesis,<sup>19</sup> a fact partially attributed to methodologic insufficiencies of the published studies. Another possible theory is that patients with more hostile tumors develop symptoms earlier, seeking medical attention sooner; nevertheless, these patients still have to face a more grievous outcome, because these malignancies display a more aggressive biologic behavior.

**Miscellaneous.** Comorbid conditions may worsen disease outcome as a consequence of increased organic stress afflicting the patient.<sup>12,13,20</sup> Survival rates are lower in patients with concomitant OSCC and disorders such as congestive heart failure, arrhythmias, peripheral vascular disease, pulmonary and renal diseases, and other cancers, either treated or untreated.<sup>20</sup> Depressed host immune status seems to play an adverse role on survival of patients with oral cancer. Patients under immunosuppressive therapy following solid organ transplant

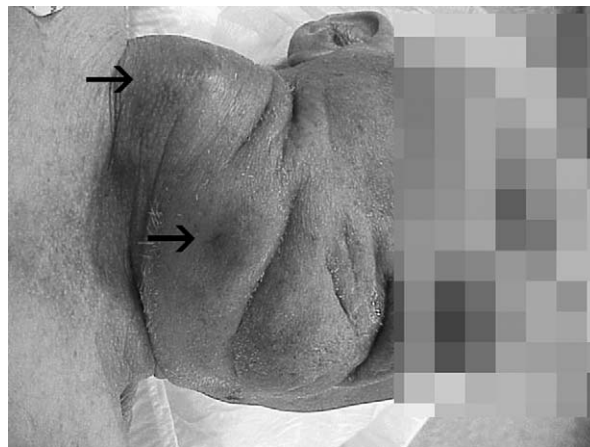


Fig. 2. Exuberant late cervical metastases from previously treated squamous cell carcinoma of the inferior lip (photograph taken with the patient's consent).

who developed OSCC fare worse than individuals with a less depressed immune system.<sup>21</sup> Additionally, there is an association between lower 5-year survival rates in patients with OSCC and evidence of immune depression.<sup>22</sup> These facts highlight the importance of immune response in tumor control and the potential value of cancer immunotherapy.<sup>22,23</sup> Some specific symptoms, such as odynophagia, oral abnormal hemorrhage, and weight loss, also have been correlated with shorter survival.<sup>12</sup> Weight loss is associated with higher mortality rates in patients with OSCC with recurrence or persistence of disease, or second primary tumor.<sup>9</sup>

### Tumor-related factors

**Anatomic site.** Vascular and lymphatic networks, which vary between different anatomic sites, may influence tumor evolution and the outcome. Higher metastatic disease rates for SCC at the base rather than at the oral tongue have been reported.<sup>24</sup> Leite and Koifman<sup>10</sup> showed higher mortality rates in patients with tongue carcinomas than in those who developed lip carcinomas. In addition, some anatomic sites are linked with poorer outcome owing to the rich lymphatic drainage and the local extension being hard to evaluate and manage, such as the superior gingivolabial sulcus.<sup>25</sup>

**Disease staging.** Cancer staging is based on the TNM system, which has been labeled as imperfect, per se, for prognostic purposes.<sup>8,9,12,13,26-30</sup> However, the vast majority of authors accepts that disease staging has a crucial influence on the outcome. Guerra et al.<sup>31</sup> reported 5-year survival rates of 82% for initial stages and 49% for advanced disease. Lo et al.<sup>7</sup> described 5-year survival rates of 75%, 65.6%, 49%, and 30% for disease stages I, II, III, and IV, respectively. Gonzales-Moles et al.<sup>26</sup>

showed categorical influence of parameters T, pathologic T, N, and pathologic N on the vital prognosis. Nguyen and Yueh<sup>9</sup> found 1-year survival rates of 60% (stage I) and 32% (stage IV) in patients with recurrence, persistent disease, or second primary cancer.

Cervical node metastases (Fig. 2) have variable incidence and are widely accepted as one of the major prognostic factors in patients with OSCC.<sup>32-35</sup> Their presence is associated with a decrease in global survival to roughly half as well as with higher recurrence rates.<sup>24,34-36</sup> Cervical node metastases may be classified into 2 distinct categories: overt (clinical) or nonovert (occult). The latter may be further categorized as metastatic deposits detectable by traditional methods (staining with hematoxylin and eosin and observed with light microscopy) or “submicroscopic” metastases, only apparent after performing immunohistochemical or molecular analysis of the dissected lymph nodes.<sup>32</sup> These techniques seem quite promising, given that in patients with no clinical or radiologic evidence of metastatic dissemination, occult node metastases were detected in 20%-40% of cases.<sup>36</sup> A retrospective study involving 266 patients who had previously undergone surgery and cervical node dissection, revealed that 34% of those initially classified as cN0 had, in fact, occult metastatic disease (pN+).<sup>33</sup> Therefore, staging based on pathologic analysis following neck dissection should be considered, in order to identify high-risk patients who may benefit from adjuvant therapy, because those with node metastases show significantly lower survival rates than those with disease-free nodes.<sup>33,34</sup>

**Tumor thickness.** The risk of nodal metastases and mortality rates vary directly with the thickness of the primary tumor.<sup>8,11,26</sup> O-charoenrat et al. found that tumor thickness above 5 mm is a strong predictor of occult nodal metastases and should indicate an elective neck dissection.<sup>8</sup> There is evidence that tumor thickness may exercise more influence on the survival rates than factors such as clinical and pathologic staging.<sup>26</sup>

**Extracapsular spread (ECS).** Defined as extranodal extension of metastatic deposits outside the lymph node capsule (Fig. 3), ECS is a noticeably important prognostic factor, associated with higher locoregional recurrence rates, distant metastases, and lower survival rates.<sup>33,35,36</sup> Some authors report a decrease in survival rates between 29% and 60%, as well as an increase in nodal metastases rates, when ECS is observed<sup>34</sup>; others show 5-year survival rates of 21% in patients with ECS vs 64% for those with intranodal metastases.<sup>35</sup> A descriptive evaluation system of ECS extension subdivides it into macro- and microscopic.<sup>36</sup> Macroscopic ECS is evident to the naked eye, and microscopic ECS is only demonstrable during histologic analysis. By studying the cervical nodes of 173 patients diagnosed with

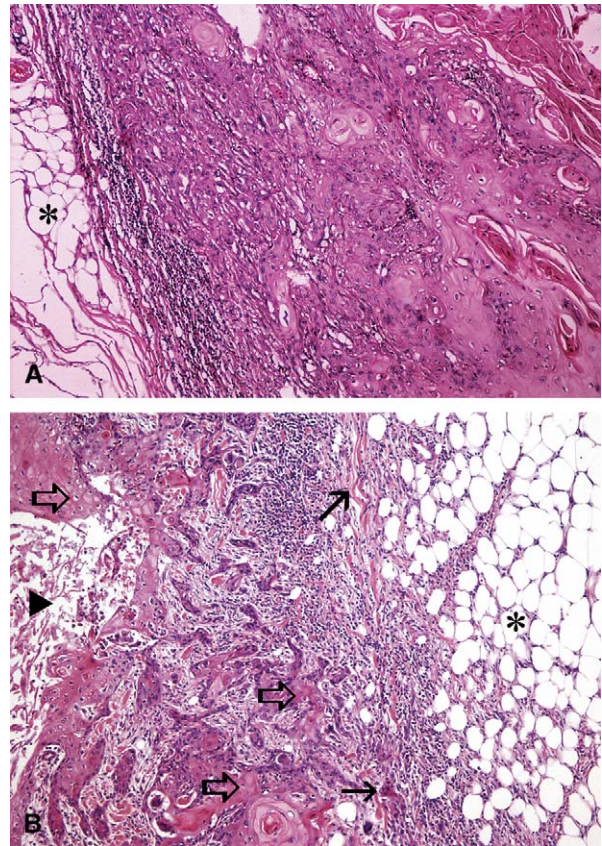


Fig. 3. **A**, Lymph node metastasis from well differentiated OSCC. There is lymph node capsule thinning and invasion (although not complete destruction). Asterisk marks perinodal fat tissue. H&E, original magnification  $\times 50$  (courtesy of Professor Silvério Cabrita, Department of Experimental Pathology, Faculty of Medicine, University of Coimbra, Portugal). **B**, Microscopic extracapsular spread, not detectable by macroscopic examination. Lymph node metastasis from well differentiated OSCC (thick arrows), with necrotic area shown (triangle). A cluster of cancer cells is shown (thin horizontal arrow), and what seem to be remnants of the lymph node capsule are still visible (thin oblique arrow). Asterisk marks perinodal fat tissue. H&E, original magnification  $\times 100$  (courtesy of Dr. Maria José Julião, Department of Pathology, Coimbra University Hospital, Portugal).

OSCC and histologically confirmed presence of nodal metastases, Woolgar et al.<sup>35</sup> found that the 3-year survival probability was similar in those with macroscopic or microscopic ECS (33% and 36%, respectively) and much worse than the rate of 72% for those with strict intranodal metastases. Additionally, it has been found that patients with multiple metastatic nodes have poorer prognosis, and individuals with multiple nodes with ECS show an extremely short median time interval until disease recurrence as well as higher mortality rates.<sup>36</sup> These findings support the notion that these patients

are at high risk for treatment failure, and are serious candidates for adjuvant treatment intensification. In a series of 266 patients treated surgically, with or without adjuvant radiotherapy, Greenberg et al.<sup>36</sup> established the 5-year disease-specific survival rates of 88% for those classified as pN0, 65% for patients pN+/ECS-, and 48% for those pN+/ECS+. Therefore, it seems important to integrate ECS into pathologic staging systems, including microscopic ECS.<sup>35</sup> The additional costs would probably be low compared to the final benefits.

**Histologic differentiation.** Most authors have established significant correlations between lower histologic differentiation and poorer prognosis,<sup>7,9,20,27,37</sup> but others did not find such association.<sup>8,10,11</sup>

**Perineural invasion.** Perineural invasion apparently correlates with higher probability of regional and distant metastases, higher depth of tumor invasion, lower differentiation, and lower 5-year survival rates in OSCC.<sup>38</sup> Rodolico et al.<sup>39</sup> showed that perineural invasion correlates with the risk of nodal metastases.

**Angiogenesis.** Malignancies have the ability to induce growth of new blood vessels, which is important for tumor progression, aggressiveness, and ability to metastasize. It is a highly regulated and complex process.<sup>40</sup> Most authors assess tumor angiogenesis by counting the number of blood vessels (microvessel density, MVD) in tissue sections. Vessels are observed using several immunohistochemical staining techniques.<sup>41,42</sup> Other methods have been developed to evaluate angiogenesis, such as the Chalkley method and flow cytometry vessel counting.<sup>41,42</sup> Using the MVD technique, Shpitzer et al.<sup>28</sup> have studied early-stage tongue SCC angiogenesis, reporting that it may be an important factor for tumor hostility. Marked angiogenesis correlated well with the risk of nodal metastases and should probably imply a more aggressive postoperative adjuvant therapy. Moreover, OSCC angiogenesis correlates with T and N parameters and is an independent predictor of tumor recurrence and a reliable prognosticator.<sup>29</sup>

The VEGF plays a decisive role in the development of blood vessels; it is a key component in tumor angiogenesis, and 4 subtypes have been described (A, B, C, and D).<sup>43,44</sup> Shintani et al.<sup>43</sup> recently described its expression in OSCC, correlating subtypes A and B with tumor angiogenesis and subtypes C and D with the risk of nodal metastases. The latter were also frequently up-regulated in the invasive front of the tumor, indicating a possible role in the process of tumor invasion and development of metastases. Uehara et al.<sup>44</sup> found a significant correlation between the high expression of VEGF in OSCC and worse prognosis.

**Tumor expression of cyclooxygenase-2 (COX-2).** Recently, a strong correlation was found in OSCC between high COX-2 expression and higher lymph node

involvement, higher recurrence rates, and shorter disease-free survival.<sup>45</sup> Marked COX-2 expression was found in the cytoplasm of cells of the tumor invasive front and also in the cells of the surrounding stroma and vessels, indicating a putative role in tumor invasion and development of metastases.<sup>16,46</sup> Another study demonstrated that COX-2 overexpression in OSCC was associated with higher radioresistance; tumor cells treated *in vitro* with a COX-2 inhibitor showed better response to radiotherapy.<sup>47</sup> These findings can obviously have interesting and valuable prognostic and therapeutic implications.

**Molecular markers.** Cancer cells result from disruptions in circuits that regulate proliferation and homeostasis of normal cells.<sup>48,49</sup> Although various genetic changes are associated with several types of disturbances and many types of cancers, there are 6 typical modifications in cellular physiology: self-sufficiency of growth signals, insensitivity to growth-inhibitor signals, evasion of apoptosis, unlimited replicative potential, ability to promote sustained angiogenesis, and capacity to invade surrounding tissues and metastasize.<sup>48</sup>

Several genetic aberrations have been identified in OSCC, most frequently in chromosomes 3, 9, 11, 13, and 17. Among others, the inactivation of tumor-suppressor genes such as *p16* (9p21) and *p53* (17p), the overexpression of oncogenes such as *PRAD-1* (11q), and the alteration of genes involved in the metabolism of carcinogens or DNA repair seem to play a role in the carcinogenesis of OSCC.<sup>49</sup> Moreover, most oral carcinomas are telomerase-activity positive.<sup>29,50</sup> The study of these alterations is important for the characterization of cancer cells, with implications in the detection of individual and familial risk, noninvasive early diagnosis, tumor staging, therapy, and prognosis.<sup>51</sup> Some of the most thoroughly studied genetic modifications implicated in the prognosis of OSCC are summarized as follows.

**Oncogenes.** Epidermal growth factor receptor (EGFR): EGFR proto-oncogene maps to 7p13-q22, and encodes a transmembrane protein whose activation by ligands such as epidermal growth factor or transforming growth factor alpha triggers a cascade of intracellular biochemical processes involved in cellular proliferation, differentiation, migration, and antiapoptotic pathways; it seems to play a significant role in cancer cell proliferation, survival, and mobility. Its overexpression is common in many malignancies, including breast, prostate, lung, and bladder cancers, correlating with poor prognosis.<sup>29,49,52,53</sup> In OSCC it has been frequently associated with advanced T stage, diffuse tumor invasiveness, and high incidence of cervical node metastases.<sup>29</sup> Its expression has also been correlated with lower histologic tumor differentiation.<sup>54</sup> Recently published works emphasize

EGFR as an anticancer therapeutic target in OSCC, with promising results both in vitro and in vivo.<sup>55,56</sup> Research concerning cancer chemoprevention of head and neck cancer using EGFR tyrosine kinase inhibitors (gefitinib, erlotinib) is also in progress.<sup>52</sup>

*c-myc*: Gene involved in the regulation of genetic expression and cell cycle. Its overexpression is associated with loss of differentiation in OSCC, although its correlation with survival is not clear.<sup>29</sup>

**Cyclin D1**: Proto-oncogene that regulates cell cycle; its product, CCND1, phosphorylates *Rb*, promoting the transition G1→S. Cyclin D1 activity is inhibited by several tumor-suppressor genes, including the subsequently discussed *p16*, *p21*, and *p27*. The amplification and overexpression of this gene are independent prognosis factors in several tumors, including head and neck SCC.<sup>57,58</sup> Increased expression of cyclin D1 is associated with the presence of regional nodal metastases, and advanced tumor stage. Therefore, it may be a useful prognostic indicator, although some authors find these data controversial.<sup>29,50,51</sup>

**Cyclin A**: Crucial for DNA synthesis in phase S and G2→M progression, its overexpression has been correlated with poorer prognosis in several human tumors. High cyclin A indices correlate with advanced disease stage, larger tumor volume, nodal metastases, and recurrence. It has been reported that patients with more than 15% cyclin A-positive cells show a significantly shorter survival when compared with those presenting less than that value.<sup>59</sup>

**Tumor-suppressor genes. *p53***: One of the most important genes influencing human carcinogenesis; protein p53 is involved in cell cycle control, apoptosis, and the preservation of genetic stability. In carcinomas, its expression is higher in those more undifferentiated, correlating with a more burdensome prognosis; yet some published works do not confirm these data, maybe partly because the mutations precede the clinicopathologic changes.<sup>29,50,51,60-62</sup> *p53* gene mutations may be better predictors of recurrence than the expression of the protein, and serum *p53* levels may be more efficient prognosticators than its tissue immunodetection.<sup>51</sup> The impact of *p53* in the prediction of tumor radiosensitivity has been investigated with contradictory results.<sup>29,50,63</sup> *p53* is an attractive target for gene therapy; some experiments have been conducted using adenoviral vectors, and encouraging results have been achieved.<sup>29</sup>

***Rb***: Its mutation or decrease of activity causes uncontrolled cellular proliferation. In OSCC the relation with prognosis is not well established.<sup>29</sup> However, Takes et al.<sup>64</sup> reported an association between loss of expression of gene *Rb* and higher probability of nodal metastases in OSCC.

***p16***: Halts cell cycle progression at G1. Deletions of *p16* seem to be crucial for the malignant progression, and deletions of 9p21 influence survival, recurrence rates, and the presence of nodal metastases. Allelic imbalance at 9p21 predicts poorer prognosis.<sup>29,51</sup>

**p21<sup>WAF1/CIP1</sup>**: An inhibitor of cyclin-dependent kinases, arresting cell cycle progression. Protein p21 is a product of genes *WAF1*, *CIP1*, or *SD11*. The expression of p21<sup>WAF1/CIP1</sup> inversely correlates with parameter T and clinical staging but not with parameter N, tumor differentiation, or apoptotic variables. Patients with tumors expressing higher values of p21<sup>WAF1/CIP1</sup> have longer disease-specific survival.<sup>65</sup>

**p27<sup>kip1</sup>**: Inhibitor of cyclin-dependent kinases, hampering the transition G1→S; its low expression has been correlated with worse prognosis in several human tumors, including OSCC.<sup>66</sup>

**p34<sup>cdc2</sup>**: Cyclin-dependent kinase that regulates cellular entry into mitosis; it is a cell proliferation index. In p34<sup>cdc2</sup>-positive tongue SCC a significantly lower survival has been found.<sup>67</sup>

**Allelic imbalance (AI)/loss of heterozygosity (LOH)**. In OSCC, patients showing AI at 1 or more loci in 3p24-26, 3p13, or 9p21 may have mortality rates 25 times higher.<sup>68</sup> High fractional allelic loss correlates with higher recurrence and less survival. Evidence exists that AI at some specific pairs of loci is a better prognosticator than the TNM staging system.<sup>68</sup> In a study of LOH at 2q, 3p, and 21q it was shown that allelic loss in these regions is associated with the progression of OSCC and correlates with worse prognosis, particularly regarding 2q.<sup>69</sup> The molecular study of these loci may help select patients who should undergo more aggressive therapies.

**Ploidy**. Abnormal DNA content has been associated with advanced stage OSCC and other markers of poor prognosis, such as lower degree of differentiation and lymph node metastasis. It appears to be an independent prognostic factor for relapse and death; it was found useful also as a valuable differential diagnosis marker for nondysplastic oral white patches or as predictor of occult nodal metastasis.<sup>70-72</sup> However, debate has been maintained over this issue owing to the reported intratumoral heterogeneity of DNA ploidy, with some authors defending a homogeneous distribution of ploidy in the tumor maintained even in the metastasis (although ploidy has not been correlated with prognosis)<sup>73</sup> and others reporting heterogeneity and thus limited application to predict prognosis.<sup>74</sup> The study of DNA content of cells in the tumor invasive front, considered important to measure tumor aggressiveness (and therefore predict outcome), suggested an influence on disease-specific survival, especially if in conjunction with clinical findings.<sup>75</sup>

Cell proliferation markers. Several methods have been used to assess cell proliferation. Their prognostic value is still a matter of debate, because different markers have been considered relevant to prognosis in some papers whereas markers of proliferation failed to correlate with the prognosis in other papers, a fact attributed to heterogeneity of series, different anatomic locations, or other differences in methodology. Apparently, no single method will be able to predict prognosis, but eventually a combination of static and dynamic parameters of cell cycle (eg, immunohistochemistry of Ki-67/MIB1 and silver staining of argyrophilic nucleolar organizer region-associated proteins) may prove to be a helpful and inexpensive prognostic factor.<sup>29,76</sup>

Intercellular adhesion molecules. Intercellular adhesion molecules are important for tumor development, invasiveness, and appearance of metastases. Some alterations in expression and/or function are reported in OSCC, and those more frequently associated with OSCC prognosis concern E- and P-cadherins, catenins, and CD44. Several papers correlate poorer prognosis with primary tumor changes of CD44v9 phenotype, with lower expression of CD44v3, and especially with reduced expression of E-cadherin and P-cadherin.<sup>77-80</sup> E-cadherin down-regulation was attributed to promoter hypermethylation.<sup>81</sup> Additionally, nodal metastases were independently associated with decreased beta-catenin expression.

*Human papillomavirus (HPV).* There is escalating evidence of a causal association between HPV and OSCC,<sup>82-93</sup> with several studies showing that HPV is associated with increased risk of oral cancer, independently of exposure to tobacco and alcohol.<sup>84,86,88,89</sup> This association is valid for high-risk HPV, which comprises subtypes 16, 18, 33, and 35. HPV-16 may be responsible for more than 80% of HPV-positive OSCC.<sup>82,83,85-88,90,93</sup> The virus can be detected in tissues or cells using several methods, namely, biochemical, immunologic, microscopic, and molecular. Polymerase chain reaction is considered the most sensitive assay.<sup>85,86</sup>

*p53* remains the most commonly mutated gene in many common human cancers, but in a high proportion of cases lacking mutations its function is compromised by other mechanisms.<sup>83,84,92</sup> Regarding HPV, this may occur via interaction of *p53* with protein E6 encoded by the oncogenic HPV types, mainly HPV-16 and HPV-18, which results in increased ubiquitin-dependent proteolysis of *p53*.<sup>82,83,92-94</sup> The expression of wild-type *p53* in HPV-positive tumors contrasts with the higher frequency of *p53* mutation in HPV-negative cases, and this fact seems to be associated with better prognosis in patients with HPV-positive OSCC.<sup>82,83,94</sup> Recently

published data on the prevalence of HPV infection in OSCC points to rates between 15% and 30%, but this might be an underestimation; it may ascend to more than 50%.<sup>84,86,87,89,91</sup> The disparity found in the published data has been associated with variations in collection of samples, the efficiency of the detection procedure, and geographic parameters. It is not well established how HPV infects the upper airway and oral cavity, but epidemiologic evidence suggests sexual transmission,<sup>82,93</sup> because the prevalence of infection increases after the onset of sexual activity, although the presence of HPV in OSCC has not yet been robustly linked to sexual practices such as oral sex.<sup>82</sup>

The presence of HPV DNA in a significant fraction of OSCC raised the question of whether HPV tumor status affects the outcome of oral cancer. To date, the findings have been inconsistent, with some studies reporting no survival differences<sup>84,87,94</sup> and others clearly stating a reduction in death risk among patients with HPV-positive tumors when compared with those with HPV-negative tumors.<sup>82,83,88,90-92</sup> A large study conducted by Gillison et al.<sup>82</sup> has shown that patients with HPV-positive tumors had a 60% reduction in risk of death from cancer and significantly improved disease-specific survival when compared patients with HPV-negative tumors. Schwartz et al.<sup>90</sup> also found a strong association between the presence of HPV-16 DNA in OSCC and prolonged survival. These findings support the theory that HPV-positive OSCC may represent a distinct molecular, biologic, and clinical identity, supposedly associated in causal terms with HPV infection and possibly carrying better prognosis than HPV-negative cases.<sup>82,83,90</sup>

*Miscellaneous.* Uridine phosphorylase (UPase) is an enzyme that catalyzes the phosphorolysis of uridine to uracil. Its expression and activity are increased in solid tumors, including head and neck tumors. Positive UPase marking correlates well with lymphatic metastases, but not with tumor size or location, histologic differentiation, or global survival.<sup>95</sup> Protein S100A4 (involved in the mobility of cancer cells) has been associated with strong tumor invasiveness and higher probability of nodal metastasis.<sup>96</sup> The expression of the glucose transporter Glut-1 was studied in OSCC, as well as tumor glucose metabolism. High levels of both parameters correlated with a significantly shorter survival,<sup>97</sup> which is consistent with the notion that tumors with aggressive biologic behavior present higher metabolic levels. Lymphocytic infiltration surrounding invasive carcinomas is composed mainly of T lymphocytes, and represents a true immune response. The prognosis seems better for marked infiltrations than for smaller ones.<sup>27,29</sup> A strong association between OSCC auto-fluorescence (emitted by pigments of the porphyrin

group) and categories T and N has been found; therefore, autofluorescence may be an indicator of tumor progression and, eventually, prognosis.<sup>98</sup>

### Treatment-related factors

It is not our present intention whatsoever to evaluate the efficacy of the several therapeutic modalities used in OSCC. However, we should point out some issues related to the treatment of OSCC with likely influence on the outcome.

*Cervical node dissection.* Classically, the surgical procedure employed in the presence of noticeable cervical node metastases has been radical neck dissection (for details regarding cervical node classification see reference<sup>99</sup>). However, this procedure is a source of significant postoperative morbidity, namely shoulder dysfunction, which may be minimized using a 2-stage procedure. Furthermore, recently published work asserts that sparing 1 or both internal jugular veins is associated with a reduction in mortality rates, not endangering the prognosis.<sup>32</sup> Selective neck dissection, highly dependent on the primitive tumor location, apparently achieves similar regional control and survival rates as those attained with more extensive neck dissections.<sup>32</sup> This approach may also serve staging purposes and assist the selection of patients for adjuvant therapy. Sentinel node detection, using lymphocytigraphy or dye injection, may be beneficial in the choice of the type and extension of neck dissection, effectively decreasing the aggressiveness of surgical interventions. The method is apparently not difficult to implement and easily identifies the sentinel node.<sup>100,101</sup> Ross et al.<sup>100</sup> have established the sensitivity of the procedure as 94%, when using the lymphocytigraphy, the dye, and the full pathologic protocol.

*Resection margin.* A strong correlation has been demonstrated between a resection margin free of disease and higher survival rates, with longer time until recurrence of disease.<sup>11,31,35</sup>

## DISCUSSION

Despite the attainments already achieved concerning OSCC diagnosis and therapy, mortality and morbidity rates are still exceedingly high, challenging the available methods of prognosis assessment and encouraging the search for new and better markers, namely, molecular markers that relate comprehensively with known alterations of tumor progression. The immense diversity found in the field of clinical oncology must be considered from 2 main perspectives: the biologic distinctiveness of each patient and the biologic distinctiveness of each malignancy. Currently, in practical terms, the factors with greater consensual influence on disease outcome include disease staging, extracapsular spread,

tumor thickness, and resection margin free of disease. In the future, better results in clinical oncology appear to rely on improved understanding of tumor molecular biology.

A vast number of molecular markers have been correlated with OSCC outcome, illustrating the complex events leading to carcinogenesis and cancer progression. Furthermore, some of the proposed markers are frequently debated and sometimes results seem to contradict each other. Several factors may explain this situation, such as the small number of individuals included in each study or the heterogeneity of selected patients, which frequently differ in various features, notably tumor location. One other complexity is the possible intratumoral heterogeneity of the marker, for which multiple sampling of the tumor may be the key. Tumor invasive front analysis is gaining relevance because it might better reflect tumor-host interactions and consequently the aggressiveness and prognosis.

A multitude of factors are involved in prognosis, and probably no single marker can accurately predict the final results. Tumor progression is a multifactorial and multistep process; therefore multiple marker evaluation may logically be required to estimate the final results. Unfortunately, the widespread introduction of biologic markers into daily clinical practice has been slow and rather ineffective, hampering the completion of clinical studies to assess their real usefulness and facilitate their definitive implementation. In addition, the scattering of published data complicates the translation into the clinical setting.

Global RNA expression analysis can be achieved by DNA microarray technology, which has been used in various cancers,<sup>102</sup> including oral cancer,<sup>103,104</sup> to obtain gene expression profiles, associating them subsequently with clinical features. The potential of this method is vast, but one must not forget that cDNA microarray assays can only analyze the transcriptome, whereas biologic function is mediated mostly by proteins. RNA levels do not always correlate with protein levels and are not sensitive to post-translational modifications. Recently, proteomic methods such as 2-D gel electrophoresis and high-throughput mass spectrometry have been used to establish salivary proteome, and reliable information can be obtained through them. Multiple biomarker analysis can establish patterns which may be associated with the outcome.<sup>105,106</sup> However, complex patterns may be difficult to discern by the human eye and mind, and bioinformatics algorithms will probably be useful. Proteomic analysis of whole body fluid protein components has been developed for the monitoring of health status and for early disease diagnosis and characterization. In addition, salivary biomarkers, relying on a supposed link between salivary proteins and systemic

diseases, may be of value. Proteomic analysis of multiple markers present in saliva, a noninvasive and readily available method, may become in the future a powerful bedside technique.<sup>105-107</sup>

Conceivably, an upcoming all-inclusive molecular and clinical staging system will allow a more accurate selection of patients that should undergo more aggressive, specific, or individualized cancer therapy. We believe that further knowledge and subsequent application of the methods exposed above will definitely increase prognostic and therapeutic success, effectively decreasing morbidity and mortality rates associated with OSCC.

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