The prognostic role of clinical, morphological and molecular markers in oral squamous cell tumors

Minireview

Zs. NÉMETH, N. VELICH, S. BOGDAN, M. UJPÁL, G. SZABÓ, ZS. SUBA

Department of Oral and Maxillofacial Surgery, e-mail: nemeth@fok.usn.hu, Semmelweis University Budapest, H-1085 Budapest, Hungary

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Despite of considerable advances in the diagnostic and therapeutic possibilities, the prognosis of epithelial tumors in the oral cavity is still very poor. A knowledge of the prognostic factors at the begining of treatment is therefore indispensable for determination of the appropriate therapy for the given patient. These factors may be linked to the patient (e.g. age, sex, general condition and immunological parameters) or to the tumor (localization, TNM stage, histoAbbreviations: AgNOR – argyrophylic nucleolar organizer regions; AI – apoptotic index; Bcl-2 – protein which regulates the apoptosis; Cyclin-D1 – protein which regulates the cell cycle; EGF – epidermal growth factor; EGFR – epidermal growth factor receptor; ErbB-1 – a gene coding the EGF; FSH – follicle-stimulating hormon; Ki67 – nuclear antigen; MI – mitotic index; MMP – matrix metalloproteinase; MVD – capillary-density (micro vessel density); NK – natural killer; P53 – nuclear protein; PCNA – proliferating cell nuclear antigen; TGF- – transforming growth factor-; TE – testosteron; VEGF – vascular endothelial growth factor.logical features, DNA content distribution, or immunhistochemical and other parameters). A survey of the literature reveals that the TNM stage, the grade, the mode of invasion and the depth of the tumor infiltration are generally the most important factors influencing the fate of the patient. The prognosis depends primarily on the clinicopathological parameters, though even if they are known, it is not possible to screen out those patients who are at particular risk of a relapse. During the past 10 years, study of the DNA content distribution, the proliferation markers and certain oncogenes has come into the focus of attention; great interest is also shown in the extracellular matrix components and the metalloproteinases, which play key roles in the invasion and metastasis formation.

Key words: oral cancer, prognostic markers, tumor stage, survival

The prevalence of the oral cavity tumors is displaying the most dynamic growth among malignancies. Whereas the death rate from tumors overall in Hungary rose by only 2.8 fold between 1948 and 2000, that from tumors of the lip, oral cavity and pharynx in the same period increased nearly 6 fold. The increases relative to 1970 and 1980 were 4.5 fold and 2.5 fold, respectively, and even during the 1990s there was a rise of around 70 % [63]. Since nearly 70 % of the patients present for care in stage III or IV, many experts consider that the solution lies in the prevention or at least the early recognition of the disease; in narrower sense, however, this is a health-policy rather than a health-care question.

In spite of the fact that there have been substantial developments in both diagnostic and therapeutic possibilities during the past 20 years, the prognosis of epithelial cancers of the head and neck is still very unfavourable. The fate of the patients depends decisively on the classical clinicopathological parameters: location, tumor size, lymph node status, tumor grade, depth of invasion and distant metastasis [44, 60]. In many cases these factors do not ensure a clear cut standpoint as concerns the prognosis; it is known that, even in tumors of the same histological type, location and stage, the course of the disease may differ considerably in two given patients.

Abbreviations: AgNOR – argyrophylic nucleolar organizer regions; AI – apoptotic index; Bcl-2 – protein which regulates the apoptosis; Cyclin-D1 – protein which regulates the cell cycle; EGF – epidermal growth factor; EGFR – epidermal growth factor receptor; ErbB-1 – a gene coding the EGF; FSH – follicle-stimulating hormon; Ki67 – nuclear antigen; MI – mitotic index; MMP – matrix metalloproteinase; MVD – capillary-density (micro vessel density); NK – natural killer; P53 – nuclear protein; PCNA – proliferating cell nuclear antigen; TGF- – transforming growth factor-; TE – testosteron; VEGF – vascular endothelial growth factor.

During the past 30 years, great emphasis has been placed on the determination of prognostic factors in cases of malignant tumors of the head and neck. Numerous systems have been devised, and numerous investigations have been performed, not only on the clinical parameters relating to the patient and the tumor, but also on histological, immunohistochemical and biological markers.

The Department of Oral and Maxillofacial Surgery at Semmelweis University is the largest stomato-oncological centre in Hungary. 800–900 new cases of carcinoma of the oral cavity are treated annually. Based on our own treatment experiences of high number of cases as well as recent literary data we are trying to summarize prognostic values of all patient and tumor related data which could provide information already at the time of diagnosis concerning the likely course of the disease.

Patient-related factors

The significance of age in the establishment of the prognosis has been widely discussed. It is our own experience that the younger the patient, the poorer the prognosis. A number of clinical investigations have analysed the possibility of a correlation between the age of the patient and the course of the disease, but a positive correlation has not been found [17]. Similarly, no connection has been demonstrated between the age and the prognosis, or between the age and the response to chemotherapy (predictive factor) [17, 53].

The distribution of malignant tumors of the oral cavity on the basis of sex is around 3:1 for males/females. However, regression analysis of the data of COGNETTI et al on 152 patients indicated a poorer prognosis for males [13]. EIBAND found that sex and ethnicity did not have significant effects on the survival [17]. Nevertheless, it may be stated with certainty that, among our own patients with tumors of the oral cavity, the proportion of females tends to increase from year to year. This is especially true for lingual carcinoma, which was earlier observed almost exclusively in males. The current tendency may be explained by the spread of smoking and alkohol consumption.

Simultaneous alcohol consumption and smoking is a high risk factor in the development of malignant tumors of the oral cavity. The data of DIETZ and SZABÓ demonstrated that, if the relative risk factor for subjects who do not smoke or consume alcohol is taken as 1, that for smokers is 15, and that for alcoholics is 13, while it is 195 for those who smoke and drink [15, 66].

A number of studies have dealt with the possibility of a connection between the general condition of the organism and the effectiveness of chemotherapy [3, 16, 31]. The above-mentioned investigation by COGNETTI at al demonstrated a significant correlation between the general state of the patient and the response to cytostatic treatment [13].

In consequence of the heterogenity and complexity of the immune-system, the immunological components are difficult to evaluate as prognostic factors. Many observations have been made in attempts to decide wheather the effectivity of cytostatic agents depends on the absolute lymphocyte count, on the proportions of the T-cell subpopulations [59], and the natural killer (NK) cell activity, and/or on the serum levels of the various immunoglobulins [52]. Even though the results of some studies approached or even slightly exceeded the limit of sigificance, no correlation was found between the values of the measured parameters and the extent of remission.

A very important probability is the utilization of the remaining immunoreactivity of the cancer patient. Cytostatic blocking of the immune system can be prevented by an individual treatment, and a continuous control of the laboratory findings.

Disease-related factors

The anatomical location is an important (but according to the clinical examinations not significantly important) factor determining the prognosis [34]. It is well known that the survival rates are favourable in cases of lower lip tumors, and the survival is also essentially better in cases of forward location of tongue or buccal tumors than in cases involving the root of the tongue, the mesopharynx or the floor of the mouth that are in the same stage [14]. Our own observation suggests that the prognosis becomes less and less favourable in the following sequence of locations: lip, bucca, free edge of the tongue, floor of the mouth, gingiva and mesopharynx.

Among patients with tumors of the oral cavity, we may distinguish those in an early stage with a better prognosis (stages I and II) and those in a more advanced stage with unfavourable prognosis (stages III and IV). Naturally, because of the characteristics of the stage classsification, it may occur that a small tumor causing only minimal bone destruction (without cervical lymph node enlargement) may be classified in the same stage as a tumor 4 cm in diameter that gives multiple cervical metastases on one side. In our experience, in Hungary 70–80 % of the patients are in stage III-IV when they first seek out a physician, and this fact itself is responsible for the poor prognosis.

In their retrospective study of 152 patients, EIBAND et al found that there is a significant difference between patients in stage I or II and those in stage III or IV as regards the overal survival [17].

Tumor size. Similarly as for tumors in other localisations, the size of a tumor in the oral cavity can be a determining prognostic factor. The basis of the TNM stage classification is given by the largest diameter of the tumor, together with the regional and distant lymph node status. Nevertheless, in cases of tumors of the oral cavity the size of the tumor frequently does not exhibit a close correlation with the clinical course. In accordance with other authors, WOOLGAR et al concluded that the depth of the invasion in the direction of the base of the tumor is the determining prognostic factor, and not the surface dimensions [19, 73]. FRIERSON et al reported a

significant correlation between the prognosis and the depth of invasion of tumors of the lower lip. The critical depth of the infiltration was determined to be 6 mm. For tumors extending deeper than this, locoregional metastasis formation was observed in three-quarters of the patients [21]. However, the correlation between the cervical lymph node status and the prognosis is not clear cut. This is indicated by the observation of MOORE et al that there is only a low rate in occurence of metastatic cervical lymph nodes in cases of epithelial tumors smaller than 2 cm in the oral cavity, and metastatic lymph nodes are not significantly more frequent even in cases of larger tumors [42].

Lymph node status. Numerous studies have confirmed that the lymph node status is the most important prognostic factor in cases with head and neck tumors. The incidence of cervical lymph nodes associated with the tumor displays a very wide scatter, varying in a stage-dependent manner between 1 % and 75 % in different publications [5]. The survival has been reported to display a positive correlation with the number of enlarged lymph nodes, their location and their degree of mobility. A number of authors state that the prognosis primarily depends not on the number of metastatic lymph nodes, but on the tumorous infiltration of their capsule [6, 17, 66, 73].

Distant metastases. Some authors describe that 10 % of patients with tumors in the head and neck already have distant metastases at the time of diagnosis [39]. Distant metastases develop in further 10 % in the course of the disease [36]. Autopsy reveals distant metastases in 45–50 % of the cases, most often affecting the lungs, the mediastinal lymph nodes (35 %), the liver (30–40 %) and the bones [74]. In our complex of cases, distant metastases are much more rare (less than 5 %). Epithelial tumors of the oral cavity that have existed even untreated for 1–1.5 years rarely give metastasis below the level of the clavicle.

Histological parameters

Tissue differentiation. Grade. During the past nearly 80 years, numerous authors have proposed classification systems relating to the tissue differentation of squamous epithelial tumors in the head and neck region [3, 8, 9, 68, 29]. ANNEROTH determined 6 morphological features as the basis of his system: keratinization, nuclear polymorphism, mitosis rate, mode of invasion, depth of invasion, and degree of stromal lymphocyte infiltration. Each of these parameters was evaluated on a scale of 1–4, and the scores were summed. The features were studied in the least differentiated parts of the tumor [3]. The most-widespread grading system is that of THOMSON, who classified tumors into well-differentiated, moderately differentiated and poor-differentiated categories [68].

A number of authors have demonstrated that grade may be taken into consideration as an independent prognostic factor. FRIERSON et al reported that metastases are more frequent in cases of grade III or IV [21]. Other authors have found that the prognosis is very unfavourable for tumors displaying a low level of differentation [61]. A comprehensive study of 114 cases of epithelial tumors of the head and neck revealed a close correlation between a low degree of differentiation and the lymph node status. It has been shown that well-differentiated tumors give rise to cervical metastases more rarely than do moderately or poor-differentiated epithelial tumors.

The investigations by HENK and LANGDON indicated that the 5-year survival rates for well-differentiated, moderately-differentiated and undifferentiated tumors were 40 %, 26 % and 12 %, respectively [24].

Perineural spread. Perineural spread has been characterized by some authors as an indicator of aggressive tumor progression [10, 21]. BYERS et al observed a perineural spread in 20 patients with tumor of the lower lip; this was accompanied by a significantly increased incidence of cervical lymph node metastasis and by a shorter survival [10]. A perineural spread was observed by DANIELE et al in only 3 cases, but they did not find a positive correlation with the occurrence of cervical lymph node metastases [14].

Capillary density. Determination of the capillary density is a relatively simple means of mapping tumor angiogenesis. Neovascularisation, which is indispensable for the growth of tumors, involves the formation of new vessels, starting from the existing vascular network [4]. If vascular invasion is observed in a morphological examination, this is a sign of an unfavourable prognosis. On processing histological preparations from 33 patients with squamous epithelial tumors of the oral cavity, ALCADE et al observed that the capillary density on the invasive front of the tumors that gave rise to cervical metastases was significantly higher than on the corresponding areas of tumors that did not give metastases [2]. A similar conclusion was reached by other authors [62].

Apoptotic index. The apoptotic index (AI) is the percentage ratio of the number of apoptotic cells to the overall cell population. Its prognostic value is disputed. ITO et al did not found a correlation between the AI and the course of tumors of the oral cavity [28].

In cases of tongue carcinoma, NARESH et al observed a lower number of lymph node metastases and a longer disease-free survival when the AI was low [43]. In a study of epithelial tumors of the oral cavity, SHINTAI et al found that a low AI was associated with a weak ability to respond to radiation therapy [54].

Mitotic index. The mitotic index (MI) is the percentage ratio of the number of mitotic cells to the total cell population. It is an important measure of the degree of malignancy. It is known from various clinical studies that in some cases a high MI is associated with a better ability to respond to neoadjuvant chemotherapy, but in the same group of patients the 10-year survival rate may be higher among those with a low MI [1].

Cellular distribution of DNA content. There is a close correlation between the chromosome instability and the biological aggressivity of the tumor [18, 65].

The DNA content of the tumor cells may be the same as that of the normal cells (euploid), but it may also be higher or lower (aneuploid). Aneuploid cells are particulary characteristic for malignant tumors. The cellular DNA content may be suitable for characterization of the heterogeneity of tumor cells, and possibly for the revelation of subpopulations [33].

It appears that the prognosis in cases of aneuploid tumors with an elevated DNA content is poorer than for those with a lower DNA content [32, 51, 69].

In patients who underwent surgery and radiation therapy, BRADFORD et al observed that a higher DNA index was accompanied by a more unfavourable prognosis and by resistance to therapy [7].

Immunohistochemical parameters

Proliferation markers. PCNA (proliferating cell nuclear antigen) is a nuclear protein that indicates cell proliferation; it is a histological marker of the G1/S phase of the cell cycle. Its prognostic significance is still discussed: some authors consider that a high PCNA level is associated with an unfavourable prognosis, whereas others state that its level does not influence the outcome of the disease [34]. A number of authors claim that it may be a predictive factor: in the event of a high PCNA level, radiochemotherapy may be applied with greater success [7].

Ki67 is a nuclear antigen that is present in phases G1, S, G2 and M of the cell cycle, but not in G0; it is therefore an expressed proliferation marker. The multivariant investigations by LIU et al revealed a significant correlation between the enhanced expression of Ki67 and the lymph node status [37]. Other authors observed that, although its expression is enhanced in poorly differentiated tumors, it does not display a close correlation with the MI or the survival [23].

AgNOR (argyrophylic nucleolar organizer regions) are DNA regions which participate in RNA transcription and which can be well labeled via silver binding. Various authors have reported a correlation between the expression of the AgNOR and the progression of malignant tumors in the oral cavity [41, 67]. In connection with a study of the invasive front, PIFFKÓ et al draw attention to the independent prognostic value of the AgNOR [46].

Basal membrane components

Laminin. The laminin molecule is one of the fundamental components of the basal membrane. It has multiple biological roles, e.g. in cell adhesion, in the spread, migration, proliferation and differentiation of tumor cells [45, 47]. Linear deposition varying in intensity around the nests of tumor cells is characteristic. Laminin-5 is often found in the cytoplasm of squamous epithelial tumor cells of the invasive front, which suggests that there is a connection between its presence and the invasion. Its total or partial absence correlates with the unfavourable outcome of tumor diseases.

Collagen IV. Besides laminin, another main component of the basal membrane of the intact tissues (vessels and epithelium) is collagen IV, which shows up around the cell nests in epithelial tumors. It plays important roles in the development and stabilization of the tumor matrix, and hence in the inhibition of tumor invasion and metastasis formation. Matrix metalloproteinases (such as collagenase IV = MMP-2) promote progression of the tumor by loosening and desintegrating the structure of the matrix and breaking it down. Expression of these enzymes are likewise important prognostic factors [40]. The studies by SHI et al indicated that deficient production of collagen IV and a simultaneous enhanced expression of MMP-2 are associated with a poor prognosis [53].

Oncogenes, suppressor genes

P53 is a nuclear protein which participates directly in the regulation of the cell cycle. Its mutation is one of the most frequent gene mutation observed in tumor patients [32]. In the event of damage to the P53 (suppressor) gene (e.g. mutation in response to carcinogenes), the control function of the protein does not operate, as consequence of which the cell becomes genetically unstable and genetic defects accumulate. P53 builds up in the cell nucleus and can be detected by immunohistochemical reactions [34].

P53 exhibits mutation in 50–70 % of cases of epithelial tumors of the head and neck [56, 70, 71]. Some authors consider that mutation of the gene means a poor prognosis for tumors of the head and neck; others have not found a close correlation between the presence of the mutation and the course of the disease; indeed, a larger survival has been reported in the event of P53 accumulation in tumors of the root of the tongue [34, 70].

ErbB-1. Of the four members of the erbB gene family, the best known is erbB-1, which encodes EGF. The enhanced expression of the erbB oncogenes is an important determinant of the course of tumor diseases. However the prognostic value is contradictory in cases of epithelial tumors of the head and neck. It was observed by WERKMEISTER et al that amplification of the erbB oncogene exhibited a close correlation with a decreased duration of complaint-free survival [72]. In contrast, FIELD et al did not detect any correlation between the amplification of erbB and the clinicopathological parameters on the survival rates [20].

Cyclin-D1, a member of the cyclin family, is a protein that regulates the cell cycle. In agreement with other authors, MICHALIDES et al drew attention to the fact that an enhanced expression of cyclin-D1 is associated with a poor prognosis [40].

Other factors

Bcl-2: The protein bcl-2 displays an elevated expression in numerous tumors and is an important regulator of apoptosis.

It protects the cells against apoptosis induced by genotoxic agents, and accordingly is believed to play a role in the development of resistance to therapy [55]. Opinions as to its prognostic significance differ: some authors regard its elevated expression as an unfavourable prognostic sign, whereas others expect the opposite [70].

EGFR (epidermal growth factor receptor) is a transmembrane glycoproteine which plays a role in cell growth. Apart from EGF, it is able to bind TGF-α (transforming growth factor α). EGFR production is enhanced in 55–100 % of epithelial tumors of the head and neck. A number of publications have reported that the elevated expression of EGFR is closely correlated with the size of tumors, with their metastatic capacity, with the cervical lymph node status, and with the response to therapy [30, 50].

VEGF (vascular endothelial growth factor) and its receptors have an important role in the formation of new vessels [15, 57]. There is a close correlation between neoangiogenesis and the progression of tumors in the oral cavity; the prognosis of tumors that produce enhanced amounts of VEGF is essentially poorer [4].

TGF-α (Transforming Growth Factor-α) is a polypeptide which is similar in structure to EGF, and it competes with EGF for the EGFR binding sites. Its enhanced production correlates closely with the probability of development of local recurrences, and hence with the survival. The role of TGF-β1 has been investigated during the progression of tumors of the oral cavity, and it has been found that this has not prognostic value as concerns the course of the disease [38].

MMP. Important roles are ascribed to the MMP (matrix metalloproteinase) molecules as concerns the invasion of tumor and metastasis development. The enzymes have the ability to break down the components of the basal membrane and the extracellular matrix, thereby making possible the direct spread of the tumor and the metastasis formation. The best-known of them are MMP-2 (a type IV collagenase, 92 kD gelatinase B) and MMP-3 (stromielisin-1) [25, 35]. Earlier, only their role in the breakdown of the matrix was known, but it has since been recognized that they have key roles in the growth of primary and metastatic tumors.

Syndecan-1 is a cell-surface proteoglycan which can interact with a number of effector molecules (extracellular matrix molecules, growth factors, etc.). The expression of syndecan-1 is enhanced during differentation of keratinocytes, while a decreased expression may be observed in epithelial tumors [58]. In epithelial tumors, its level is proportional to the degree of tissue differentation; in poorly-differentiated tumors, it is not present or only slightly expressed. It has recently been demonstrated in stroma, its appearence being indicative of a worse prognosis [27, 48].

FSH, TE. In a study of patients with tumors of the head and neck, REMENÁR et al observed an unfavourable prognostic effect when the FSH (follicle-stimulating hormone) level was above the normal range and the TE (testosterone) level was below the normal range. However, these changes in the hormone levels could be explained in part by simultaneous liver damage characteristic in patients with tumors in the head and neck. Thus, their roles in the pathogenesis and progression of tumors require further investigations [49].

Conclusions

Summarizing the survey of the available literature data, it may be stated that the prognostic value of the classical clinicopathological parameters is often uncertain. In consequence, many patients in the oncological units may be under- or over-treated. Nevertheless, it emerges from the publications that the TNM stage, the grade and the depth of invasion of the tumor have important effects on the course of the disease. During the past 20 years, many articles have been written and many clinical observations have been made in efforts to find factors of prognostic value, but the results are often controversial.

As far as clinical practice is concerned, it would be extremely important to identify reliable markers that clearly indicate the prognosis. This would facilitate selection of the patients into homogenous groups which could then receive optimized treatment. With the aid of such markers, we could learn more about the course of the disease, and hence apply more effective treatment designed individually. In this way, unnecessary overtreatment imposing even harmful effect on the patient could be avoided.

References

[1] AKASHI-TANAKA S, TSUDA H, FUKUDA H. Prognostic value of histopathological therapeutic effects and mitotic index in locally advanced breast cancers after neoadjuvant chemotherapy. Jap J Clin Otolaryngol Abstract 1999; 193: 5–11.

[2] ALCADE RE, SHINTANI S, YOSHIHAMA Y, MATSUMURA T. Cell proliferation and tumor angiogenesis in oral squamous cell carcinoma. Anticancer Res 1995; 15: 1417–1422.

[3] ANNEROTH G, BATSAKIS J, LUNA M. Review of the literature and recommended system of malignancy grading in oral squamous cell carcinomas. Scand J Dent Res 1987; 95: 229–249.

[4] ARTESE L, RUBINI C, FERRERO G, FIORONI M, SANTINELLI A et al. Microvessel density (MVD) and vascular endothelial growth factor expression (VEGF) in human oral squamous cell carcinoma. Anticancer Res 2001; 21: 689–695.

[5] ATULA TS, GRÉNMAN R, VARPULA MJ, KURKI TI, KLEMI PJ. Palpation, ultrasound, and ultrasound-guided fine-needle aspiration cytology in the assessment of cervical lymph node status in head and neck cancer patients. Head Neck 1996; 18: 545–551.

[6] BAATENBURG DE JONG RJ, HERMANS J, MOLENAAR J, BRIAIRE JJ, LE CESSIE S. Prediction of survival in patients with head and neck cancer. Head Neck 2001; 23: 718–724.

[7] BRADFORD CR, WOLF GT, CAREY TE, ZHU S, BEALS TF. Predictive markers for response to chemotherapy, organ preser-

vation, and survival in patients with advanced laryngeal carcinoma. Otolaryngol Head Neck Surg 1999; 121: 534–538.

[8] BRODERS AC. The microscopic grading of cancer. Surg Clin North Am 1941; 21: 947–961.

[9] BRYNE M, KOPPANG HS, LILLENG R, STENE T, BANG G et al. New malignancy grading is a better prognostic indicator than Broder's grading in oral squamous cell carcinoma. J Oral pathol Med 1989; 18: 432–437.

[10] BYERS RM, O'BRIEN CJ, WAXLER J. The therapeutic and prognostic implications of nerve invasion in cancer of the lower lip. Int J Radiol Oncol Biol Phys 1978; 4: 215–219.

[11] CHAMBERS AF, MATRISIAN LM. Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 1997; 89: 1260–1270.

[12] CHOW V, YUEN APW, LAM KY. Prognostic significance of serum p53 protein and p53 antibody in patients with surgical treatment for head and neck squamous cell carcinoma. Head Neck 2001; 23: 286–291.

[13] COGNETTI FC, PINNAR P, RUGGERI EM et al. Prognostic factors for chemotherapy response and survival using combination chemotherapy as initial treatment for advanced head and neck squamous cell cancer. J Clin Oncol 1989; 7: 829–837.

[14] DANIELE E, RODOLICO V, LEONARDI V, TRALONGO V. Prognosis in lower lip squamous cell carcinoma: Assessment of tumor factors. Pathol Res Pract 1998; 194: 319–324.

[15] DIETZ A, RUDAT V, CONRADT CH, WEIDAUER H, HO A. Prognostic relevance of serum levels of the angiogenic peptide bFGF in advanced carcinoma of the head and neck treated by primary radiochemotherapy. Head Neck 2000; 22: 666–673.

[16] DRELICHMANN A, CUMMINGS G, AL-SARRAF M. A randomized trial of the combination of cisplatinum, oncovin and bleomycin (COB) versus methotrexate in patients with advanced squamous carcinoma of the head and neck. Cancer 1983; 52: 399–403.

[17] EIBAND JD, ELIAS EG, SUTER CM, GRAY WC, DIDOLKAR MS. Prognostic factors in squamous cell carcinoma of the larynx. Am J Surg 1989; 158: 314–317.

[18] ÉLÖ J, SEBÖK J, VARGA L. Proliferation activity of laryngeal tumors and its relation to metastases. Fül Orr Gégegyógy 1991; 37: 24–29 (In Hungarian).

[19] FANG FM, LEUNG SW, HUANG CC, LIU YT, WANG CJ et al. Combined-modality therapy for squamous carcinoma of the buccal mucosa: treatment results and prognostic factors. Head Neck 1997; 19: 506–512.

[20] FIELD JK, SPANDIDOS DA, YIAGNISIS M. C-erb-e expression in squamous cell carcinoma of the head and neck. Anticancer Res 1992; 12: 613–620.

[21] FRIERSON HF, COOPER PH. Prognostic factors in squamous cell carcinoma of the lower lip. Hum Pathol 1986; 17: 346–354.

[22] GASPARINI G, BEVILACQUA P, BONOLDI E, TESTON YT, WANG CJ et al. Predictive and prognostic markers of patients with head and neck squamous cell invasive carcinoma treated with concurrent chemoradiation therapy. Clin Cancer Res 1995; 1: 1375–1383.

[23] GONZALEZ-MOLES MA, CABALLERO R, RODRIGUEZ--ARCHILLA A. Prognosis value of the expression of Ki-67 for squamous cell carcinoma of the oral cavity. Acta Stomatol Belg 1996; 93: 159–165.

[24] HENK and LANGDON. Malignant tumours of the oral cavity. London, Edward Arnold, 1985.

[25] HEREDIA A, BURTIN P. Collagenases and cancers. Pathol Biol (Paris) 1998; 36: 1147–1150.

[26] HILL BT, PRICE LA, MACRAE K. Importance of primary site in assessing chemotherapy response and 7-year survival data in advanced squamous-cell carcinomas of the head and neck treated with initial combination chemotherapy without cisplatin. J Clin Oncol 1986; 4: 1340–1347.

[27] INKI P, KUJARI H, JALKANEN M. Syndecan in carcinomas produced from transformed epithelial cells in nude mice. Lab Invest 1992; 66: 314–323.

[28] ITO T, FUJIEDA S, TSUZUKI H, SANAGA H, FAN G et al. Decreased expression of Bax is correlated with poor prognosis in oral and oropharyngeal carcinoma. Cancer Lett 1999; 140: 81–91.

[29] JACOBBSON PA, ENEROTH CM, KILLANDER D, MOBERGER G, MARTENSSON B et al. Histologic classification and grading of malignancy in carcinoma of the larynx (a pilot study). Acta Radiol Ther Phys Biol 1973; 12: 1–8.

[30] KE LD, ADLER-STORTHZ K, CLAYMAN GL, YUNG AWK, CHEN Z. Differential expression of epidermal growth factor in human head and neck cancers. Head Neck 1998; 20: 320–327.

[31] KISH JA, WEAVER A, JACOBS J, CUMMINGS G, AL-SARRAF M. Cisplatin and 5-Fluorouracil infusion in patients with recurrent and disseminated epidermoid cancer of the head and neck. Cancer 1984; 53: 1819–1824.

[32] KOELBL O, ROSENWALD A, HABERL M et al. p53 and Ki-67 as predictive markers for radiosensitivity in squamous cell carcinoma of the oral cavity? An immunohistochemical and clinicopathological study. Int J Radiat Oncol Biol Phys 2001; 49: 147–154.

[33] KOPPER L, JENEY A, editors. Onkologia. From the gene to the hospital bed. Medicina, 2002 (In Hungarian).

[34] KRAXNER H. Prognostic factors in head and neck lymph node cancer and their role in therapy planing. PhD Thesis. Semmelweis University Medical School, 2002; 361 (In Hungarian).

[35] KUSUKAWA J, SASAGURI Y, SHIMA I, KAMEYAMA T, MORIMATSU M. Production of matrix metalloproteinase 2 (gelatinase/type IV collagenase) and 3 (stromelysin) by cultured oral squamous cell carcinoma. J Oral Pathol Med 1992; 21: 221–224.

[36] LEEMANS CR, TIWARI R, NAUTA JJ, VAN DER WAAL I, SNOW GB. Regional lymph node involvement and its siginficance in the development of distant metastases in head and neck carcinoma. Cancer 1993; 71: 452–456.

[37] LIU M, LAWSON G, DELOS M, JAMART T, IDE C. Predictive value of the fraction of cancer cells immunolabeled for proliferating cell nuclear antigen or Ki67 in biopsies of head and neck carcinomas to identify lymph node metastasis: comparison with clinical and radiologic examinations. Head Neck 2003; 25: 280–288.

[38] LOGULLO AF, NONOGAKI S, MIGUEL RE, KOWALSKI LP, NISHIMOTO IN et al. Transforming growth factor β1 (TGFβ1) expression in head and neck squamous cell carcinoma patients as related to prognosis. J Oral Pathol Med 2003; 32: 139–145.

[39] MERINO OR, LINDBERG RD, FLETCHER GH. An analysis of distant metastasis from squamous cell carcinoma of the upper respiratory and digestive tracts. Cancer 1977; 40: 145–151.

[40] MICHALIDES R, VAN VEELEN N, KRISTEL P, HART AA, LOFTUS BM et al. Overexpression of cyclin D1 indicates a poor prognosis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 1997: 123: 497–502.

[41] MIGALDI M, CRISCUOLO M, ZUNARELLI E, LO BIANCO L, MARTINELLI BM et al. p120 and AgNOR nucleolar protein expression. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85: 189–196.

[42] MOORE C, FLYNN MB, GREENBERG RA. Evaluation of size in prognosis of oral cancer. Cancer 1986: 58: 158–162.

[43] NARESH KN, LAKSHMINARAYANAN K, PAI SA, BORGES AM. Apoptosis index is a predictor factor of metastatic phenotype in patient with early stage squamous carcinoma of the tongue: hypothesis to support this paradoxical association. Cancer 2001; 91: 578–584.

[44] OLASZ L, SZABÓ I, HORVÁTH A. A combined treatment for advanced oral cavity cancers. Cancer 198; 62: 1267–1274.

[45] PATEL V, ALDRIDGE K, ENSLEY JF, ODELL E, BOYD A et al. Laminin-γ_2 overexpression in Head and Neck squamous cell carcinoma. Int J Cancer 2002; 99: 583–588.

[46] PIFFKO J, BÁNKFALVI Á, ÖFNER D, BRYNE M, RASH D et al. Prognostic value of histobiological factors (malignancy grading and AgNOR content) assessed at the invasive tumour front of oral squamous cell carcinoma. Br J Cancer 1997; 75: 1543–1546.

[47] PIRILA E, SHARABI A, SALO T, QUARANTA V, TU H et al. Matrix metalloproteinases process the laminin-5 gamma2-chain and regulate epithelial cell migration. Biochem Biophys Res Commun 2003; 18: 303: 1012–1017.

[48] PULKKINEN JO, PENTTINEN M, JALKANEN M, KLEMI P, GRENMAN R. Syndecan-1: A new prognostic factor in laryngeal cancer. Acta Otolaryngol (Stockh) 1997; 117: 312–315.

[49] REMENÁR É, SZÁMEL I, BUDAI B, OROSZ Z, GAUDI I et al. Prognostic significance of sex steroids and hypophyseal hormones in head and neck squamous carcinoma. Magyar Onkológia 2003; 47: 155–159 (In Hungarian).

[50] RESNICK JM, UHLMAN D, ADAMS G, GAPANY M, ADAMS G et al. Cervical lymph node status and survival in laryngeal carcinoma: prognostic factors. Ann Otol Rhinol Laryngol 1995; 104: 685–694.

[51] RUÁ S, COMINO A, FRUTTERO A, CERA G, SEMERIA C et al. Relationship between histologic features, DNA flow cytofotometry, and clinical behaviour of squamous cell carcinomas of the larynx. Cancer 1991; 67: 141–149.

[52] SCHANTZ SB, GOEPFERT TH. Mutimodal therapy in distant metastasis: The impact of natural killer cell activity. Arch Otolaryngol Head Neck Surg 1987; 113: 1207–1213.

[53] SHI H, HE R, LIN G. Role of type IV collagene and type IV collagenase in the invasion and metastasis of salivary adenoid cystic carcinoma (Abstract). Hua Xi Kou Qiang Yi Xue Za Zhi 1997; 15: 218–219.

[54] SHINTANI S, MIHARA M, NAKAHARA Y, TERAKADO N, YOSHIHAMA Y et al. Apoptosis and p53 are associated with effect of preoperative radiation in oral squamous cell carcinomas. Cancer Lett 2000; 154: 71–77.

[55] SILVESTRINI R, VENERONI S, DAIDONE MG. The bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph-node negative breast cancer patients. J Natl Cancer Inst 1994; 86: 499–504.

[56] SISK EA, SOLTYS SG, ZHU S, FISHER SG, CAREY TE et al. Human papillomavirus and p53 mutational status as prognostic factors in head and neck carcinoma. Head Neck 2002; 24: 841–849.

[57] SMITH BB, SMITH GL, CARTER D, SASAKI CT, HAFFTY BG. Prognostic significance of vascular endothelial growth factor protein levels in oral and oropharyngeal squamous cell carcinoma. J Clin Oncol 2000; 18: 2046–2052.

[58] SOUKKA T, POHJOLA J, INKI P, HAPPONEN RP. Reduction of syndecan-1 expression is associated with displastic oral epithelium. J Oral Pathol Med 2000; 29: 308–313.

[59] STROME M, CLARK J, FRIED M. The prognostic implications of defining natural killer cell function and T-cell subsets in patients with squamous cell carcinoma. In: Fee W, Goepfert H, Jones M et al, editors. Head and Neck Cancer. Philadelphia, BC Decker 1990: 89–93.

[60] SUBA ZS, SZABÓ GY. A new histologic scoring system characterizing the effect of intraarterial chemotherapy. Reg Cancer Treat 1991; 43: 4.

[61] SUBA ZS, SZABÓ GY, TÓTH-BAGI Z. Tissue specimen examination and its relation to the prognosis of oral squamous cell carcinoma. Fogorv Szle 1993; 86: 297–303 (In Hungarian).

[62] SUBA ZS, TÓTH-BAGI Z. Relation of the oral carcinoma capillary density to the effectivity of intraarterial chemotherapy. Magyar Onkológia 1994; 38: 161–164 (In Hungarian).

[63] SUBA ZS. Role of the histology parameters in complex therapy of oral carcinomas. PhD Thesis, Budapest 1994.

[64] SUBA ZS, BARABÁS J, TÓTH-BAGI Z. The effect of intraarterial chemotherapy in oral cavity lymph node metastases. Fogorv Szle 1995; 88: 331–338 (In Hungarian).

[65] SUDBO J, BRYNE M, JOHANNESSEN AC, KILDAL W, DANIELSEN HE et al. Comparison of histological grading and large scale genomic status (DNA ploidy) as prognostic tools in oral dysplasia. J Pathol 2001; 194: 303–310.

[66] SZABÓ G, KLENK G, VEÉR A, NEMETH Z. Correlation of the combination of alcoholism and smoking with the occurence of cancer in the oral cavity. A screening study in an endangered population. Mund-Kiefer Gesichts Chir 1999; 3: 119–122.

[67] TEIXEIRA G, ANTONANGELO L, KOWALSKI L, SALDIVA P, FERRAR A et al. Argyrophylic nucleolar organizer regions staining is useful in predicting recurrence-free interval in oral tongue and floor of mouth squamous cell carcinoma. Am J Surg 1996; 172: 681–683.

[68] THOMSON ST CL. The history of cancer of the larynx. J Laryngol 1939; 54: 61–87.

[69] TYTOR M, GEMRYD P, GRENKO R, LUNDGREN J, LUNDQUIST PG et al. Adenoid cystic carcinoma: significance of DNA ploidy. Head Neck 1995; 17: 319–327.

[70] VENERONI S, SILVESTRINI R, COSTA A, SALVATORI P, FARANDA A et al. Biologic indicators of survival in patients treated by surgery for squamous cell carcinoma of the oral cavity and oropharynx. Oral Oncology 1997; 33: 408–413.

[71] WARNAKULASURIYA S. Lack of molecular markers to predict

malignant potential of oral precancer. J Pathol 2000; 190: 407–409.

[72] WERKMEISTER R, BRANDT B, JOOS U. The erbB oncogenes as prognostic markers in oral squamous cell carcinomas. Am J Surg 1996; 172: 681–683.

[73] WOOLGAR JA, SCOTT J. Prediction of cervical lymph node metastasis in squamous cell carcinoma of the tongue/floor of mouth. Head Neck 1995; 17: 463–472.

[74] ZBÄREN P, LEHMANN W. Frequency and sites of distant metastases in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1987; 113: 762–764.