# Prognosis value of the expression of Ki-67 for squamous cell carcinoma of the oral cavity

M.A. GONZALEZ-MOLES[1], R. CABALLERO[2], A. RODRIGUEZ-ARCHILLA[1], I. RUIZ-AVILA[3], I. BRAVO[4]

[1] *Department of Oral Medicine. School of Dentistry. University of Granada (Spain).*

[2] *Department of Oral Medicine. School of Dentistry. University of Barcelona (Spain).*

[3] *Pathologist. Jaen General Hospital (Spain).*

[4] *Doctor at Intensive Care and Emergency Service. Hospital Virgen de las Nieves. Granada.*

**Abstract**

**Ki-67 is a nuclear antigen expressed in $G_1$, S, $G_2$ and M phase of cell cycle and absent in quiescent cells ($G_0$). In some neoplasms, Ki-67 expression has a prognostic value. The purpose of this study was to determine the role of Ki-67 expression like prognostic factor in oral squamous cell carcinoma (OSCC). Monoclonal antibody MIB-1 that recognizes Ki-67 antigen was used. 74 OSCCs were analyzed. 49% of OSCCs did not express Ki-67 antigen. In the Ki-67 positive tumours, the expression was slight in 36.5%, moderate in 10.8% and intense in 10.8% of the cases. In all the positive OSCCs, the distribution of the marking was patchy in different zones of the tumour, moreover, in 65% of the lesions, the positive cells were located mainly in the proximity of intraoral blood vessels. A significantly more intense expression was noted on tumours that had not been differentiated ($p<0.05$), with a larger nuclear pleomorphism ($p<0.05$) and in lesions that invaded in the form of disassociated neoplasic cells of in small groups of neoplasic cells ($p<0.001$). However, the expression of Ki-67 did not correlate with the mitosis count and it had no influence on survival.**

KEY WORDS

**Ki-67 antigen; oral squamous cell carcinoma; prognosis.**

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a frequently occurring tumour, which represents 4% of malignant neoplasms in men and 2% in women [8,10]. It is located in an easily explored site and this reason should be sufficient to justify excellent results with respect to early diagnosis of the tumour leading to a good therapeutic response. However, this is not usually the case and a rather high number of tumours are diagnosed at advanced stages. At a result, aggressive treatments are required, altering the patient's future quality of life, yet they are not effective enough to reduce the mortality rate for this cancer (50% 5-year survival rate) [8]. Therefore, one of the most interesting aspects in the study of this kind of neoplasms is knowledge about tumour prognosis which would lead to a specific adjustment to the therapy and a reduction in the hypothetically unjustified therapeutic excesses together with the financial burden they entail. Clinical and pathological parameters have

normally been used to make an assessment on tumour prognosis. These parameters are all of a semi-quantitative nature and, generally speaking, they are not at all precise in their prognosis, often failing to find a biological parameter to indicate the aggressiveness of the tumour [9]. The growth of a tumour is primarily dependent on the fraction of growth cells, its speed of growth and the fraction of neoplasic cells that die [5]. Therefore, the study of the parameters that highlight the proportion of tumourous cells that are splitting, could theoretically provide data on the growth fraction of a specific neoplasia and perhaps have important implications for prognosis purposes. From all the procedures that analyse the cellular kinetics of a population of neoplasic cells, the detection of antigens that are expressed in proliferating cells, using immunohistochemical techniques that use monoclonal antibodies, have the advantage of being able to be to stored tissues and allow the performance of retrospective studies for application to the analysis of cancer patients' survival rates. Furthermore, they are simple and speedy techniques which provide results that are comparable to other methods for studying cellular kinetics [6, 11]. Amongst the monoclonal antibodies used in the study of cellular kinetics there are those that recognize the proliferating cell nuclear antigen (PCNA) and the Ki-67 antigen. The PCNA is not expressed throughout the whole cellular cycle. It is first detected in the G1 phase, reaches its maximum expression in the S phase or in G1/S, it falls in the G2 phase and becomes impossible to detect by immunohistochemical methods in the M phase [13]. The Ki-67 antigen is expressed in G1, S, G2 and M and some studies have demonstrated that the marking rate for Ki-67 is relevant for prognosis purposes in some human tumours (2).

The aim of this study is to determine the value of the expression of Ki-67 for prognosis purposes in the OSCC.

## MATERIALS AND METHODS

A study was carried out on a group of 74 patients, 68 men and 6 women aged between 40 and 85 (mean age 62.4), diagnosed and treated for squamous cell carcinoma at the University Hospital of Granada (Spain). All the cases complied with the criteria established by the Department of Oncology.

In order to obtain a sample with unbiased results, a random group was selected from a list of clinical records for 50 patients with lip carcinoma and 50 with carcinoma of the oral cavity who had been diagnosed and treated before 1992.

Patients were eligible for the study if they met the following criteria:

(i) Patient had been diagnosed and treated for squamous carcinoma of the oral cavity.

(ii) Patient had finished the initial treatment before 1992.

(iii) Patient had a clinical history and a section of the paraffin block of the primary tumour was available.

Of the 100 patients who initially seemed to meet the study criteria, 26 were excluded and 74 finally took part in the study.

In all the cases an assessment of the clinical history was made. Previous case history and clinical manifestations of importance were collected (general health; general manifestations of the tumour: weight loss and anorexia; tumour location; macroscopic aspect; size of the primary tumour; involvement of cervical lymph nodes; distant metastases; clinical stage).

The status concerning relapse was assessed with the following parameters: months following relapse; final status of the patient (possibilities: signs of relapse; no signs of disease with relapse controlled; the patient lives with the disease; death caused by oral cancer. From the perspective of the survival analysis, the first three variables are classified as survival and the last one as death); months of survival.

In addition, a section of the paraffin block of the primary tumour was obtained and stained with haematoxylin-eosin, which was used for the histopathological analysis (degree of differentiation; degree of keratinization; nuclear pleomorphism; number of mitoses; inflammatory infiltrate; tumour infiltration pattern).

Five-micron sections of the biopsy specimens were deparaffinized, rehydrated, and incubated with 0.05% trypsin in TBS (tris buffered saline) for 20 minutes at 37°C. After enzyme digestion, slides were rinsed in TBS. Antigen retrieval was then performed by treating the slides for 15 minutes at 750 w in citrate buffer (10 mM; pH 6.0) in a microwave oven. After washing in TBS, sections were blocked with normal rabbit serum for 30 minutes and incubated at room temperature for 60 minutes with

MIB-1 monoclonal antibody (Inmunotech, Marseille, France) specific for Ki-67 antigen diluted 1:100 in TBS. The slides were then washed in TBS and incubated at room temperature for 30 minutes with a biotinylated rabbit anti-mouse monoclonal antibody (1:200) (Dakopatts, Denmark). After another washing step, antibodies were located using the avidin-biotin complex immunoperoxidase technique, applying a commercial Elite ABC kit (Vectastain, Vector laboratories, Burlingame, Calf., USA). Finally the sections were slightly counterstained in Mayer's haematoxylin and mounted in an aqueous mounting medium. 500 cells were counted in different fields of the tumour, with the expression of Ki-67 being evaluated as absent (0% of marked nuclei), slight (up to 5% of nuclei marked), moderate (between 5% and 30% of nuclei marked) and intense (over 30% of nuclei marked).

The following statistical procedures were used:

a) Descriptive Statistics

Arithmetical mean, standard deviation and percentages.

b) Analytical Statistics

- Chi-square test for comparison of several sample proportions. The Yates correction in 2 x 2 tables. Fisher's exact test was used whenever any of the expected values was below 5.
- The Spearman rank correlation method with quantitative, ordinal and dichotomic variables.
- Survival analysis using the actuarial method [4]. The survival time as a dependent variable (time up to disease-related death) was used whenever the full survival time was unknown, since at the last time of contact, the patient was not yet in the terminal phase of the disease. The yearly accumulated survival percentage was estimated (with standard deviation). The relationship between different variables and mortality was studied and variables were dichotomized following clinical and scientific criteria by means of the Lee-Desu test. A list of the potential variables related to mortality is to be found in Table 1.

The possibility of confusion or interaction was assessed by using the Cox regression analysis model [4]. Due to its well known influence on mortality, the primary site of the tumour was forced (lower lip and others). Some of the variables (Table 1) were dichotomized using the partial method [4] and were included in the model as statistically significant ($p < 0.05$, in accordance with similarity ratio).

TABLE 1
Classification of the potential variables for assessing morbidity (n=74)

| Variable | Categories |
|---|---|
| Age (years) | Up to 59<br>Over 60 |
| Sex | Male<br>Female |
| Smoking | Non-smoker<br>Smoker |
| Drinking habits | Non-drinker<br>Drinker |
| Primary site | Others<br>Lower Lip |
| Size of the primary tumour (T) | T1/T2<br>T3/T4 |
| Node metastases (N) | No<br>Yes |
| Clinical stage | I<br>II/III/IV |
| Degree of differentiation | WDSCC<br>MDSCC/PDSCC |
| Keratin production | Low/moderate<br>Intense |
| Nuclear pleomorphism | Grade I (low)<br>Grade II (moderate)/Grade III (intense) |
| Number of mitoses | 0-5/8 fields / 40 x<br>>5/8 fields / 40 x |
| Inflammatory infiltrate | Low/moderate<br>Intense |
| Pattern of tumour infiltration | Solid<br>Cordal/small aggregates |
| Ki-67 expression | Negative/mild<br>Moderate/intense |

WDSCC: Well-differentiated squamous cell carcinoma.
MDSCC: Moderately-differentiated squamous cell carcinoma.
PDSCC: Poorly-differentiated squamous cell carcinoma

The variables were analyzed using the SPSS Version 6.01 for Windows computer program (SPSS Inc. Chicago, Illinois) [12].

## RESULTS

The clinical results of the present study are shown in Table 2. It must be pointed out that almost all tumours found in this study manifested themselves as exophytic masses or ulcers (36.5% and 58.1%). At the time of the diagnosis, tumour size amounted to a diameter of less than 2 cm in half of the cases, cervical lymph nodes were not frequently found (16.3%) and there were no distant metastases In the authors' sample, 14.9% of the patients presented late stages (III and IV) when diagnosed.

TABLE 2
Clinical manifestations of the tumours (n=74)

| Variable | Distribution | n (%) |
|---|---|---|
| General health [a] | No | 57 (95.0) |
| | Yes | 3 (5.0) |
| General manifestations [b] | No | 44 (61.1) |
| | Yes | 28 (38.9) |
| Site of primary tumour | Base of tongue | 9 (12.2) |
| | Lateral margin | 7 (9.5) |
| | Floor of mouth | 4 (5.4) |
| | Retrocomisures | 1 (1.4) |
| | Lower lip | 45 (60.8) |
| | Upper lip | 3 (4.1) |
| | Comisures | 2 (2.7) |
| | Palate | 1 (1.4) |
| | Base of tongue and larynx | 2 (2.7) |
| Macroscopical aspect [a] | Exophytic | 27 (36.5) |
| | Endophytic | 1 (1.4) |
| | Ulcerous | 43 (58.1) |
| | Verrucous | 3 (4.1) |
| Size of primary tumour (T) | T1 | 37 (50.0) |
| | T2 | 14 (18.9) |
| | T3 | 8 (10.8) |
| | T4 | 15 (20.3) |
| Node metastases (N) | N0 | 62 (83.8) |
| | N1 | 6 (8.1) |
| | N2a | 3 (4.1) |
| | N2b | 3 (4.1) |
| Distant metastases | M0 | 74 (100) |
| | M1 | 0 (0) |
| Clinical stage | I | 40 (54.1) |
| | II | 23 (31.1) |
| | III | 10 (13.5) |
| | IV | 1 (1.4) |

a: unknown in 14
b: unknown in 2

TABLE 3
Pathological parameters of 74 tumours

| Variable | Distribution | n (%) |
|---|---|---|
| Degree of differentiation [a] | WDSCC | 34 (49.3) |
| | MDSCC | 27 (39.1) |
| | PDSCC | 8 (11.6) |
| Keratin production [a] | Minimal | 9 (13.0) |
| | Moderate | 21 (30.4) |
| | Intense | 39 (56.5) |
| Nuclear pleomorphism [b] | Grade I (low) | 36 (52.9) |
| | Grade II (moderate) | 22 (32.4) |
| | Grade III (intense) | 10 (14.7) |
| Number of mitoses [a] | 0-1/8 F/40x | 38 (55.1) |
| | 2-5/8 F/40x | 13 (18.8) |
| | >5/8 F/40x | 18 (26.1) |
| Inflammatory infiltrate [b] | Low | 9 (13.2) |
| | Moderate | 26 (38.2) |
| | Intense | 33 (48.5) |
| Tumour infiltration [b] | Solid | 30 (44.1) |
| | Cordal | 25 (36.8) |
| | Small aggregates of tumour cells | 13 (19.1) |

a: unknown in 5
b: unknown in 6
F: fields

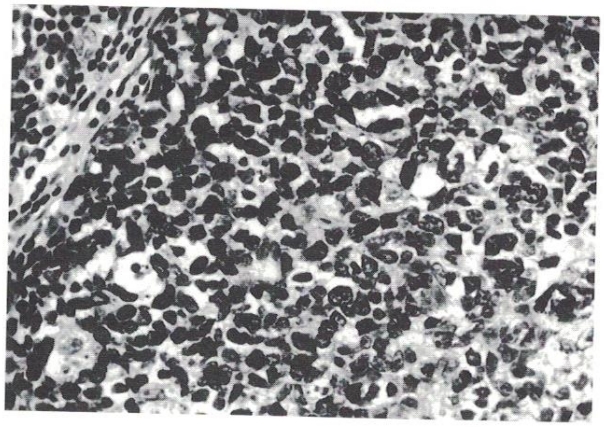

Fig. 1
Intense expression of Ki-67 antigen in OSCC sample.

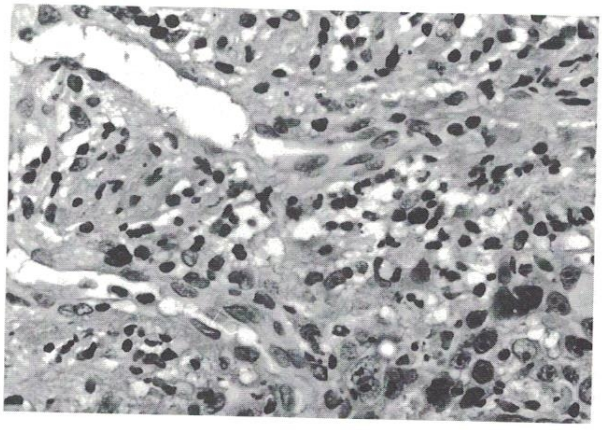

Fig. 2
Perivascular expression of Ki-67 antigen.

The results from the histopathological study are listed in Table 3. It is important to highlight the good or moderate distinction between tumours in these series (49.3% and 39.1%).

Forty-one point nine per cent of the tumours did not express Ki-67 antigen. In the Ki-67 positive tumours, the expression was slight in 36.5%, moderate in 10.8% and intense in 10.8% of the cases (Fig. 1). In all the positive tumours, the distribution of the marking was patchy in different zones of the tumour, moreover, in 65% of the lesions, the positive cells were located mainly in the proximity of intraoral blood vessels (Fig. 2). A significantly more intense expression was noted on tumours that had not been differentiated ($p<0.05$), with a larger nuclear pleomorphism ($p<0.05$) and in lesions that invaded in the form of disassociated neoplasic cells or in small groups of neoplasic cells ($p < 0.001$). However,

TABLE 4
Description of survival for 74 patients with oral cancer

| Time of survival (months) | Number of patients; At the beginning of the interval/censured/ at death | Accumulated survival proportion (ee) [a] |
|---|---|---|
| 0-<12 | 72/0/2 | 0.972 (0.019) |
| 12-<24 | 70/1/9 | 0.846 (0.043) |
| 24-<36 | 60/0/2 | 0.818 (0.046) |
| 36-<48 | 58/0/1 | 0.804 (0.047) |
| 48-<60 | 57/0/0 | 0.804 (0.047) |
| 60-<72 | 57/2/0 | 0.804 (0.047) |
| 72-<84 | 55/5/0 | 0.804 (0.047) |
| 84-<96 | 50/2/0 | 0.804 (0.047) |
| 96-<108 | 48/14/0 | 0.804 (0.047) |
| 108-<120 | 34/32/0 | 0.804 (0.047) |

a: standard error
Mean survival is above 120 months

the expression of Ki-67 did not correlate with the mitosis count.

The survival rate among the 74 patients studied at 12-month intervals is shown in Table 4. The overall survival rate after 60 months stood at 80% with a small standard deviation (0.047). The mean survival time for patients with cancer in our study was 120 months. In the assessment of these results both intraoral and lip cancers were considered. Table 5 shows the influence of the different variables on patient survival with oral cancer. The variables which most influence survival

TABLE 5
Influence of different variables on survival of 74 patients with oral cancer

| | Cumulative survival ± ee | | Comparison[a] z, p |
|---|---|---|---|
| Variable, categories | A 36 months | A 60 months | |
| Site of primary tumour | | | |
| Others | 0.546±0.097 | 0.507±0.008 | z=22.327 |
| Lower lip | 0.978±0.022 | 0.978±0.022 | p<0.001 |
| Size of primary tumour (T) | | | |
| T1/T2 | 0.980±0.020 | 0.980±0.020 | z=31.764 |
| T3/T4 | 0.455±0.106 | 0.409±0.105 | p<0.001 |
| Node metastases (N) | | | |
| No | 0.885±0.041 | 0.885±0.041 | z=12.326 |
| Yes | 0.455±0.150 | 0.364±0.145 | p<0.001 |
| Clinical stage | | | |
| I | 1.000±0.000 | 1.000±0.000 | z=21.500 |
| II/III/IV | 0.586±0.088 | 0.554±0.089 | p<0.001 |
| Degree of differentiation | | | |
| WDSCC | 0.909±0.050 | 0.909±0.050 | z=3.954 |
| MDSCC/PDSCC | 0.739±0.075 | 0.709±0.078 | p<0.05 |
| Nuclear pleomorphism | | | |
| Grade I | 0.914±0.047 | 0.914±0.047 | z=5.240 |
| Grade II/III | 0.714±0.081 | 0.681±0.083 | p<0.05 |

a: Lee-Desu Test, with 1 grade of freedom; z: contrast, p: statistical significance. The survival curves of the two categories considered in each variable are compared. For simplicity, only survival after 36 and 60 months are included.
F: fields.

TABLE 6
Cox regression analysis model in 74 patients with oral cancer

| Variable | ß (ee) | $e^{ß}$ | Wald Test, p |
|---|---|---|---|
| Primary site | | | |
| Lower lip (reference) | 1.6 (1.203) | 5.08 | Wald=1.84, p=0.174 |
| Others | | | |
| Size of primary tumour (T) | | | |
| T1/T2 (reference) | 2.7 (1.205) | 15.65 | Wald=5.26, p<0.05 |
| T3/T4 | | | |

were location (p<0.001), primary tumour size (p<0.001), involvement of cervical lymph nodes (p<0.001), clinical stage (p<0.001), degree of tumour differentiation (p<0.05) and nuclear pleomorphism (p<0.05). The cumulative survival and error standards were recorded 36 and 60 months after the evolution of the clinical stage. It is worthwhile pointing out that the expression of Ki-67 had no influence on survival.

Once the site variable was forced (lower lip) in the Cox multiple regression model as a consequence of the known influence of labial site on survival, it was noted that the variable which affected survival to the greatest extent was the primary tumour size (p<0.05) (Table 6).

## DISCUSSION

The behaviour of the expression of the Ki-67 antigen was, in many aspects, what was expected. Thus, a greater expression was observed in tumours that had not been differentiated (p<0.05), in lesions with a larger nuclear pleomorphism (p<0.05) and in the ones that invaded adjoining tissues through cell structures that were not very cohesive (p<0.001). However, an absolute lack of statistical association was observed between the expression of the Ki-67 antigen and the mitosis count. This aspect would seem to be contradictory if the behaviour of Ki-67 is taken into account throughout the cell cycle. The Ki-67 antigen is a marker for cells in proliferation, whereas it is not expressed in cells in the quiescent G0 phase [5]. In this sense, some hypotheses may be put forward for explaining the lack of any statistical relationship between the number of mitosis and the expression of Ki-67. Thus, just like other authors [5], a large percentage of positive tumours have been found for Ki-67 in which cells close to blood vessels were marked and in every case the positive test appeared by following a patchy pattern all over the tumour tissue. In

our opinion, this finding is of tremendously importance and possibly limits the validity for determining this antigen as a definite factor for prognosis. We believe that its perivascular and patchy distribution is indicative of a dependency on local factors for its expression, possibly of a nutritional nature, which may well explain the appearance of cells in negative mitosis for Ki-67. The insufficient supply of nutrients to the neoplasic cells would result in a delay in the change-over in the cell cycle with an accumulation of cells in the G0 phase and ultimately cell death [18]. As a result of the swelling of the vascular wall structure in the intratumoural vessels and the lack of proper lymphatic drainage, the deep vessels in a tumour may collapse due to a high extravascular pressure [3], which may possible offer the reason for the high percentage of negative neoplasic cells for Ki-67. This observation may alter the concept that has been held till now about the distribution of the antigen in the cell cycle and, in this sense, we believe that in order for its expression to be manifested in proliferating cells, the latter must be subjected to favourable local factors, possibly of a nutritional nature.

These findings also suggest the existence of two different cell clones in a tumour that are representative of quiescent and proliferating fractions. Nevertheless, although it would seem logical to think that not all the cells in a neoplasia necessarily have to be in the proliferating phase, this hypothesis does not explain one of the observations in our study, which has also been made by other authors [5], which is the complete absence of the expression of Ki-67 in 41.9% of the tumours in our series (31 cases), and so, it would appear logical to think that the expression of Ki-67 responds, as has been said, to other factors, since the absence of a proliferating compartment in a tumour goes against the concept of neoplasic disease. Neither may it be excluded that the specific nature of the antigen is low in cells that split up slowly and in cell populations that grow in less than optimum conditions [15] and on the basis thereof, it could be argued that the number Ki-67 + cell may not necessarily reflect the proportion of the tumour that grows under these conditions.

On the other hand, Gerdes *et al.* [7] described the kinetics of the expression of Ki-67 in cell lines and demonstrated their association with protein synthesis, although not with the replication of DNA. This may also be used as an argument to explain the lack of any statistical correlation in our test between the expression of Ki-67 and the count for the mitosis figures, since both functions (protein synthesis and replication of DNA) may occur separately in different cell populations in a simple lesion and explain the potential differences in the expression of Ki-67 [1].

Finally, it is also most interesting to point out the lack of influence of the expression of Ki-67 on tumour survival rates (p=0.839). No conclusive data are to be found in the literature on this subject as regard oral cancer, though results are to be found for other sites with a different signification. Victorzon *et al.* [16], after analysing a large number of gastric carcinomas, did not observe any influence of the expression of the Ki-67 antigen on the survival rate after five years, hence they conclude that this parameter is of no value for prognosis purposes. On the other hand, Volt *et al.* [17] observed a significantly lower rate of Ki-67 marking in survivors of cutaneous melanomas than those who did not survive and Tsuji *et al.* [14] found that patients with urinary bladder cancer, whose tumour samples had a higher rate of Ki-67, had a significantly worse prognosis than those with a lower rate.

To sum up, according to our results no relevance may be attributed to the expression of the Ki-67 antigen in the prognosis for OSCC, whereas the influence of some clinical parameters was indeed significant (location, tumour size, involvement of cervical lymph nodes and clinical state) and that of pathological parameters (grade of differentiation and nuclear pleomorphism). The diagnosis of small-sized tumours was the factor, out of all of them, that had the most favourable effect on survival.

**REFERENCES**

[1] AL-SALEH, W., *et al.* – Assessment of Ki-67 antigen immunostaining in squamous intraepithelial lesions of uterine cervix. *Am. J. Clin. Pathol.*, 1995; *104*: 154-160.

[2] BROWN, D.C., GATTER, K.C. – Monoclonal antibody Ki-67: its use in histopathology. *Histopathology*, 1990; *17*: 489-503.

[3] DENEKAMP, J. – Review: angiogenic attack as a therapeutic strategy. In: *Gray Laboratory Annual Report*, Northwood, Great Britain, 1989, pp. 6-13.

[4] DOMENECH, J.M., SARRIA, A. – Analysis of survival. Gráficas SIGNO S.A., Barcelona, 1993.

[5] EDSTROM, S.S. *et al.* – Proliferative pattern of head and neck cancer. *Am. J. Surg.*, 1991; *162*: 412-16.

[6] GARCIA, R.L. *et al.* – Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissues. Comparison with flow cytometric analysis. *Am. J. Pathol.*, 1989; *134:* 733-39.

[7] GERDES, J. *et al.* – Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int. J. Cancer,* 1983; *31:* 13-20.

[8] HINDLE, I., NALLY, F. – Oral cancer: a comparative study between 1962-67 and 1980-84 in England and Wales. Br. Dent. J., 1991; *170:* 15-20.

[9] MANNING, M., GOLDSMITH, I. – Cytometric and pathologic indicators of survival. In: FEE, W.E., GOEPFERT, H., JOHNS, M.E., STRONG, E.W., WARD, P.H. (eds.). Proceedings of the International Conference on Head and Neck Cancer, July 31-August 5, 1988. B.C. Decker Inc., Boston, pp. 69-74.

[10] MOLLER, H. – Changing incidence of cancer of the tongue, oral cavity and larynx in Denmark. *J. Oral. Pathol. Med.,* 1980; *18:* 224-29.

[11] NAKAE, S. *et al.* – Analysis of proliferative activity using anti-proliferating cell nuclear antigen antibody in colorectal cancer. *J. Surg. Oncol.,* 1995; *60:* 149-53.

[12] NORUSIS, N.J. - SPSS for Windows. Release 6.0. SPSS Inc., Chicago, 1993.

[13] TSAI, S.T., JIN, Y.T. – Proliferating cell nuclear antigen (PCNA) expression in oral squamous cell carcinomas. *J. Oral. Pathol. Med.,* 1995; *24:* 313-15.

[14] TSUJI, M. *et al.* – Prognostic value of Ki-67 antigen and p53 protein in urinary bladder cancer: immunohistochemical analysis of radical cystectomy specimens. *Br. J. Urol.,* 1997; *79:* 367-72.

[15] VAN DIERENDONCK, J.H. *et al.* – Nuclear distribution of the Ki-67 antigen during the cell cycle: comparison with growth fraction in human breast cancer. *Cancer. Res.,* 1989; *49:* 2999-3006.

[16] VICTORZON, M. *et al.* – Ki-67 immunoreactivity, ploidy and S-phase fraction as prognostic factors in patients with gastric carcinoma. *Oncology,* 1996; *53:* 182-91.

[17] VOGT, T. *et al.* – p53-protein and Ki-67 antigen expression are both reliable biomarkers of prognosis in thick stage I nodular melanomas of the skin. *Histopathology,* 1997; *30:* 57-63.

[18] WESTIN, T. *et al.* – Tumor cytokinetic effects of acute starvation versus polyamine depletion in tumour bearing mice. *Cytometry,* 1991; *12:* 628-35.

**Address for correspondence**
*Prof. Miguel Angel González Moles*
*Medicina Oral. Facultad de Odontología*
*Colegio Maximo. Campus Universitario de Cartuja.*
*E-18071 GRANADA (Spain)*
*Phone number: (34) (58) 24 38 04*
*Fax number: (34) (58) 24 40 85*